ABSTRACT

Annual Meeting Symposia

JPS Satellite Symposium

Company Organized Symposia

Young Scientist Symposia

Joint Symposium with the Japanese Medical Science Federation

Joint Symposium with the Physiological Society of Japan

Joint Symposium with the Japanese Society of Toxicology

Symposia

1-AS-01-1 Proposal of Drug Rescuing

Yoshinori Fujiyoshi

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Pharmaceutical strategy was dramatically changed in 2000, when Britain's giant Pharmaceutical companies started to discuss about a merger. They tried to change the traditional approach to drug discovery, screening thousands of chemical compounds to find one useful drug, to modern strategy, namely evidence-based drug development based on modern biology. This change might be the dawn of the Structure-Guided Drug Development (SGDD). The success ratio of drug development is, however, still very low and the cost is also up to billion dollars. Many lead compounds have to be thrown away because of adverse effects and/or other reasons. Structural information is very useful for designing chemical compounds as SGDD. More importantly, structure analysis of the target protein and its ligand complex could act as base camp for challenging again to design better drug compounds avoiding the adverse effects. We can find essential parts for pharmacologic action as well as the parts giving no effect for the action. We can modify these parts for challenging to rescue the drug chemical avoiding the adverse effects. I named this strategy "Drug Rescuing" and we are making efforts to develop basic technologies for "Drug Rescuing".

1-AS-01-2 Development of software for computer-aided drug design—Easy CADD system for all beginners—

Yoshifumi Fukunishi

molprof, AIST

We have been developing software for computer-aided drug design for 15 years. The computer software named myPresto consists of compound database (including 42 million compounds), molecular dynamics simulation, prediction of ligand-binding pocket of protein, protein-compound docking program, virtual screening programs, drug design tools, prediction of LogS/LogP, and prediction of synthetic accessibility. The easy GUI programs of myPresto are available (MoDesk [http://www.moldesk.com/] and MF myPresto [http://www.fiatlux.co.jp/product/lifescience/MFMYPRESTO/MFmyPresto-index.html]). The integrated system named MolSpace (http://www.level-five.jp/company/) is a workstation including the GUI software MolDesk and a cloud computing controller MolGate and the users can purchase chemicals, synthesize new design compounds and examine them by in-vtro assay through e-mail. Among many important aspects of CADD tools, I focus on the progress MD simulation and the remained problems. One of the problems is the halogen atoms. Halogen is negative charged atom, but halogens bind oxygen atoms. The other problem is metal ions. The metal-ligand interaction is complexed. I will discuss about these topics.

1-AS-01-3 Pharmacogenomics-based individualization of drug therapy

Taisei Mushiroda

RIKEN Center for Integrative Medical Sciences

Knowledge of pharmacogenomics (PGx) can help to improve prediction of drug responses for establishment of individualized drug therapy that aims to provide a right drug at appropriate dosage for each individual patient. In the USA, PGx information about genomic biomarkers that can predict risk of adverse drug reactions (ADRs) and initial dosage setting is available in labels of 166 FDA-approved drugs. In particular, the US FDA recommends genotyping of polymorphisms in drug-metabolizing enzymes and HLA prior to drug administration for prevention of severe ADRs for several drugs, such as irinotecan, atomoxetine, carbamazepine and abacavir. Genome-wide association studies (GWAS) have been successful in identifying genes/SNPs associated with drug responses in the world. We have conducted and are conducting the GWAS using DNA samples from Biobank Japan, which started in 2003 for the collection of genomic DNA, serum and clinical information from about 300,000 Japanese patients, and revealed genomic biomarkers associated with drug efficacy and risk of severe ADRs in Japanese. In order to apply the identified genomic biomarkers to clinical therapeutics, further prospective clinical trials will be necessary for validation of clinical utility.

2-AS-01-1 Pharmaceutical regulatory authority's challenge for encouraging innovative drug development

Kazuhiko Mori

The Ministry of Health, Labour and Welfare

In October 2015, The Ministry of Health, Labour and Welfare designated 6 new investigational drugs (IND) which are being developed by pharmaceutical company as "SAKIGAKE designated products". These products are developed in Japan and remarkable clinical effects is shown for the first time in the world, are expected. The project which builds a disease registry centering on a national center and a clinical study core hospital has started as an infrastructure building for efficiency of clinical development of a new medicine. We expect that the clinical study for which a disease registry was used and clinical development of new drugs and new medical devices are promoted. The world still has many patients waiting eagerly for realization of a remarkable new medicine for the disease which has no effective curatives. It's requested that industry-academia-government cooperate and work on creation of a safer and more effective new drug more promptly aggressively to respond to the expectation from a patient. I'd like to introduce that the challenge in which the Ministry of Health, Labour and Welfare also aids new drug development innovation has been begun.

2-AS-01-2 AMED: Mission and vision for medical innovation

Makoto Suematsu

Japan Agency for Medical Research and Development

AMED has been started in order to fast-track medical R&D and to improve a quality of life for people. Among a diversity of different medical researches, AMED has chosen a field of rare and intractable diseases to tackle with a number of obstacles including rigid and inflexible funding systems, and "Balkanization" of mindsets in academia and researchers. There are several important reasons for launching Initiative for Rare and Undiagnosed Diseases (IRUD), as the first national project, which aims to spread a concept of sharing of data and expensive equipments, global phenotype coding for saving patients in foreign countries, and microattribution that should be recognized by all project leaders. AMED has joined in International Rare Disease Consortium (IRDiRC) in order to share many experiences in rare disease researches which were accumulated over 40 years in Japan. In parallel with IRUD, AMED has started up projects supporting venture companies developing orphan drugs and those aiming to utilize patient-derived iPS cells for mining new therapeutic interventions. I would present an overview of our missions and vision for medical innovations, and introduce drug discovery innovation & screening consortium (DISC) and a new project for activating regulatory sciences and big-data sciences.

2-AS-01-3 Regulatory Science for Translation of Cell-Based Therapeutic Products

Yoji Sato

Div. Cell-Based Thrp. Prod., National Institute of Health Science

Development of regenerative/cellular therapies using cell-based therapeutic products (CTPs) is keenly anticipated in Japan, because of difficulties in securing human organs and tissues in our country. To facilitate translation of novel CTPs, the Japanese Diet recently passed the Regenerative Medicine Promotion Act, as well as the Pharmaceuticals and Medical Devices Act (the Revised Pharmaceutical Affairs Law) and the Regenerative Medicine Safety Act. However, the development of CTPs is still uncertain, because they include advanced and emerging technologies with little clinical experiences. One of the biggest problems is that evaluation tools and approaches to ensure their safety, efficacy and quality are lacking, which affects not only the risk assessment/management of clinicians, developers and regulators, but also their risk communication with the public. This is one of the major reasons why regulatory science is needed for CTPs. At the session, I would like to overview current scientific challenges for the development of CTPs, and introduce our study to establish new methods for risk assessment of CTP-derived tumor formation, as an example of regulatory science researches.

2-AS-01-4 New strategy for evaluating safety and effectiveness of drug metabolites

Shinsaku Naito

Research and Development Center, Otsuka Pharmaceutical Factory, Inc.

A new strategy for assessing safety of metabolites is discussed to deliver high-quality drugs to patients as quickly as possible. In drug development, safety in humans is predicted through methods such as metabolite profiling and pharmacological activity screening, clinical pharmacokinetic studies, and sometimes safety studies using metabolite reference standards. In most cases, it is crucial to analyze metabolites using human samples obtained in clinical studies and to determine whether unique human metabolites are major metabolites. However, these tasks often constitute a rate-limiting factor in drug development, and drug risk-benefit considerations may require selecting the optimal strategy, utilizing PMDA consultations. In rare cases, risks associated with reactive metabolites should be screened with human samples, which would allow a comparison of the findings on reactivity in humans to those in animals. Safety evaluation of metabolites cannot readily be based on a single theoretical framework, and basically a case-by-case approach is called for. In summary, eliminating studies for unnecessary metabolite safety assessment is essential for prompt supply of high-quality drugs to the medical frontline.

2-AS-02-1 A trend of the education of nursing science and future problem

Shinobu Saito

Ministry of Education, Culture, Sports, Science and Technology-Japan

The number of schools of nursing in Japan is 241 in 2015. And the total of the entrance to school capacity is 20, 814 people. Since the education such as the nurses who had high nature was necessary, schools of nursing, graduate schools of nursing increased after the establishment of the law about the promotion of the securing of talented people such as nurses in 1992. Ministry of Education, Culture, Sports, Science and Technology was aimed for quality improvement of the nursing practice in March, 2011 and devised "nursing practice ability and arrival target for first degree". I will show some examples of the curriculum of school of nursing. And I would like to examine the knowledge that a student should learn for nursing science education.

2-AS-02-2 Pharmacology education and research and human resource development in nursing

Yumi Katano

Division of Theoretical Nursing, Yamagata University School of Medicine

Drugs can be a risk depending on their usage. Many nurses handle drugs without fully realizing this fact. Currently, more than 250 nursing colleges have been established in Japan, but colleges that treat pharmacology as a required course are not common. Thus, enhancement of pharmacology education is awaited. I would like to request the fostering of personnel who have the capacity to provide pharmacology education among graduates from nursing colleges and the guarantee of positions to accommodate them. Based on my experience in teaching physiology and pharmacology at a nursing department, I will introduce pharmacology education and research, as well as relevant human resource development, that would foster nurses who are well-versed in pharmacotherapy.

2-AS-02-3 Pharmacology from the point of view of Nursing Science: what do we make a request to Pharmacology

Keiko Yamaguchi

Dept. Nursing Nihon Fukushi Univ.

Nurses are responsible for carrying out the drug treatment of the patients under the guidance of the doctor and they monitor the patients how the drug takes an effect to the patients. Therefore, it is necessary that nurses are involved with the subject under the correct understanding of drug treatment.
Pharmacology education in nursing is a compulsory subject. Even though students learn drug therapy in lectures, exercise and training in each specialized courses in nursing, their acquisition is not quite enough because they feel difficulty to study pharmacology. They encounter many difficulties to deal with the patients after they get a job as a nurse.
What kind of knowledge and assessment skills regarding pharmacology in nursing will be required? How can we implement pharmacology in basic nursing education? We would like to discuss these matters in this symposium from the view of pharmacology education.

2-AS-02-4 Nursing Science from the point of view of Pharmacology: what can we do for Nursing Science

Toshihiko Yanagita

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The installation of nursing colleges/universities has rapidly increased from the late 1990s, there are 248 institutions in Japan as of 2015 and it is expected to reach 280 institutions by 2021. Pharmacology and clinical pharmacology education in undergraduate and postgraduate nursing colleges/universities is highly important, however, the lack of human resources involved in pharmacology education due to the rapid increase of nursing colleges/universities cannot be denied.
From the perspective of Pharmacological Society, (traditionally) physicians and pharmacists has been main roles in Pharmacology. Nurses give medicine directly to the patients and observe the therapeutic effect or side effect at the bedside. Therefore, nurses' involvement in education and research of pharmacology is expected to further development of pharmacological society.
In this topic, we would like to present mutual understanding of the nursing colleges and Pharmacological Society, re-examine the pharmacology education in nursing, as well as to seek a nursing pharmacology education corresponding to the needs of the new phase, the future of nursing education in pharmacology and the development of human resource in pharmacology education.

2-AS-03-1 Intertissue communications in glycometabolism and endothelial function

Tsuneo Kobayashi, Kumiko Taguchi, Takayuki Matsumoto

Dept. Physiol. and Morphol., Inst. Med. Chem., Hoshi Univ.

Recent studies have strongly implicated involvement of G protein-coupled receptor kinase 2 (GRK2) in the development and/or progression of cardiovascular diseases and insulin resistance. The hydrodynamic injection is an effective and simple method to control hepatic expression of transgenes in mice (e.g., systemic administration of siRNA). Indeed, our study demonstrated that the expression of GRK2 was decreased in liver but not in other organs including skeletal muscle, heart, pancreas, and spleen of diabetic mice treated with GRK2 siRNA by single hydrodynamic injection. The injection of GRK2 siRNA in diabetic mice resulted in decreased plasma levels of glucose, and improved the glucose/insulin intolerance. The injection of GRK2 siRNA also could improve the insulin-induced relaxation associated with increased Akt and eNOS activities in aortae from diabetic mice. Thus, hepatic regulation of GRK2 plays an important role in normalizations of insulin resistance and endothelial dysfunction in diabetes. In this symposium, we will describe the pathophysiological role of GRK2, especially related to NO production, in insulin resistance and diabetes, and provide novel insights into the potential field of translational investigation to treat diabetic complications.

2-AS-03-2 Role of the inter-organ neural network from the liver in systemic energy metabolism

Tetsuya Yamada, Hideki Katagiri

Dept. Metab. and Diabetes, Tohoku Univ. Sch Med.

The body equips inter-organ system consisting of negative feedback mechanism. For example, we found that neural transmission from the PPARγ overexpressing fatty liver increases energy consumption in brown adipose tissue (BAT) through sympathetic activation (Science 2006). On the other hand, mechanisms enabling (1) efficient storing of ingested energy as fat ; and (2) minimum wasting of stored fat during energy deprivation ; are necessary to maintain fat storage. We have identified the mechanisms described in (1). When excess energy intake activates the hepatic glucokinase, the vagal transmission from the liver to the brain decreases energy expenditure in BAT and leads to fat accumulation (Cell Metab. 2012). As an example of (2), we recently discovered that administration of SGLT2 inhibitors in mice decreased BAT thermogenesis due to decreased sympathetic activity, which was not present after selective hepatic vagotomy, indicating that neural signals from the liver play an important role in this mechanism. Neural networks from the liver to BAT enable appropriate fat storage and must be advantageous to mammals experiencing frequent starvation, but may cause obesity and make it difficult to recover from it in modern age of plenty.

2-AS-03-3 Brain-heart-kidney network controls homeostasis and pathology in the heart

Ichiro Manabe

Dept. Cardiovasc. Med., Univ. Tokyo Grad. Scho. Med.

Heart failure (HF) is a complex clinical syndrome characterized by cardiac function that is insufficient to meet systemic demand. Nearly half of chronic HF (CHF) patients also have chronic kidney disease (CKD), which increases their rate of cardiovascular mortality, suggesting cardiorenal linkage via mechanisms still poorly understood. We found that pressure overload in the heart activates renal collecting duct (CD) epithelial cells via sympathetic nerves. Within the kidneys, activated communication between CD cells, tissue macrophages and endothelial cells leads to secretion of CSF2, which in turn stimulates cardiac-resident Ly6Clo macrophages essential for the myocardial adaptive response to pressure overload. We show that CD-specific deletion of the transcription factor Klf5, renal sympathetic denervation or adrenergic beta2 receptor blockade/deletion disrupts the renal response to cardiac pressure overload. Our results clearly demonstrate that dynamic interplay between the heart, brain and kidneys is necessary for proper adaptation to cardiac stress, and highlight the novel homeostatic functions of tissue macrophages and the sympathetic nervous system, which represent potential targets for future CHF and CKD treatment strategies.

2-AS-03-4 Development of post-ischemic glucose intolerance induced by cerebral neuronal damage through communication system between brain and peripheral tissues

Shogo Tokuyama

Dept. Clini. Pharm., Sch. Pharmaceu. Sci., Kobe Gakuin Univ.

The communication system between brain and peripheral tissues through the autonomic nervous system is important for maintaining glucose and energy metabolism. Recently, we have found that the development of post-ischemic glucose intolerance is one of the triggers of ischemic neuronal damage. In this symposium, I would like to discuss about the mechanisms on the development of post-ischemic glucose intolerance focusing on communication system between brain and peripheral tissues. Especially, we focused the effect of orexin-A, one of neuropeptides and well known as regulatory factor of the communication between brain and peripheral tissues, on the development of the post-ischemic glucose intolerance and neuronal damage through the activation of hypothalamus-medulla oblongata-vagus nerve axis. In conclusion, our evidences support valuable insight for the advanced treatment of cerebral stroke and add to the existing literature about the role of post-ischemic glucose intolerance and the communication between the brain and peripheral tissues as one of the new therapeutic target for use in ischemic stroke patients.

2-AS-04-1 Next-generation cardiac safety assessment using human iPS cell technology: JiCSA activities

Yasunari Kanda[1,2]

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Drug-induced QT prolongation is a major cause of ventricular tachycardia such as torsade de pointes (TdP). The hERG blockade potential has been used in the non-clinical evaluation of potential TdP risk. Human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs), which express multiple ion channels, are expected to assess cardiac safety. We have organized Japan iPS Cardiac Safety Assessment (JiCSA) to generate functional iPS-CMs and validate the usage of iPS-CMs. We have evaluated cardiac differentiation protocol to overcome line-to-line variability. We also successfully generated mature hiPS-CMs by overexpressing KCNJ2, that are responsible for stabilizing the resting membrane potentials. Furthermore, we prepared standardized protocol to measure field potential of iPS-CM sheet with multielectrode array. The hERG blockerE4031 resulted in field potential prolongation and early afterrepolarization. We are now assessing proarrhythmic potential of 60 compounds to examine the TdP predictivity using iPS-CMs. In the symposium, we will present our strategy for the fit-for-purpose cells and the standardized protocol, and discuss proarrhythmic risk using iPS-CMs.

This research is supported by AMED 15mk0104053h0101 (to Yuko Sekino).

2-AS-04-2 Progress in applications of iPSC derived neurons for neuronal maturation

Tomoaki Shirao

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Differentiated neurons from human induced pluripotent stem cells (hiPSC-derived neurons) are expected to be useful for developing novel methods of treatment for various neurological diseases or drug discovery. However, the detailed process of functional maturation of hiPSC-derived neurons remains poorly understood. In this talk, I will first introduce the study comparing hiPSC-derived neurons with rat primary cultured neurons. Morphological development of rat and human neurons before axonal polarization was similar. However, axonal growth speed was slower in the hiPS neurons. We elucidated that glial conditioned medium contained a factor accelerating the axonal growth. The latter half of my talk, I will introduce the recent advancement in the application of hiPSC-derived neurons for the safety pharmacology.

2-AS-04-3 Fabrication of iPSC-derived Cardiomyocyte construct by Scaffold-free Bio 3D-Printer

Koichi Nakayama[1], Ken-ichi Arai[1], Atsuko Ojima[2]

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Inspired from bone fracture treatments in orthopedic surgery, we have established a simple method to fabricate 3D scaffold-free cell construct.This method use spheroids and temporal fixator which enable placement of various types of three-dimensional cells into desired xyz positions without need of hydrogels or biochemical reactive materials. We also developed a robotic system for scaffold-free cell construction, named "Bio 3D Printer". With this system, we already successfully fabricated living cell only construct such as cartilage, liver, cardiomyocyte, blood vessel, and so on. In this symposium,we`d like to introduce our "Heart PJ", which use iPSC-Derived Cardiomyocyte and the Bio 3D-Printer to fabricate cell only construct,which may useful for cardio-drug research tool and also future heart regeneration.

2-AS-04-4 3 Case studies where merging plastics sciences with microfabrication to serve biomedical applications we have contributed to platform technologies in life-cell-isolation, -imaging and -cultivation

Georg Bauer

Sony DADC BioSciences GmbH

Microfluidic chips often are composed of a clear polymer about the size of a credit card, and contain microfluidic channels, valves and filters replacing a whole workflow in the laboratory. They may be coated by iPS cells-allowing researchers to recapitulate the physiological and mechanical functions of the organs, and to observe what happens in real time. The environment of iPS cultivation contributes strongly to the degree to which the iPS reactivity is representative for the physiological context. In other applications the target is to identify and monitor biomarkers and to speed up the pharmaceutical development process by providing more predictive and useful measures of the efficacy and safety of new drugs in humans and at a fraction of the time and costs associated with traditional lab automation or animal testing. We will present three case studies where we have contributed to the development of novel platform technologies for life-cell-isolation, -cultivation and -imaging or provide the sample prep for DNA sequencing.

3-AS-01-1 Basics and practices of genome editing-examples from development to application of TALEN and CRISPR systems

Tetsushi Sakuma

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Programmable nucleases such as TALENs and CRISPR/Cas9 have enabled next-generation techniques for genetic engineering, called "genome editing". G "genome editing". Genome editing technology is based on the introduction of site-specific DNA double-strand break (DSB), followed by various DSB repair mechanisms including non-homologous end-joining (NHEJ), homologous recombination (HR), and microhomology-mediated end-joining (MMEJ). Basically, error-prone NHEJ or MMEJ induces short insertions and deletions at the target site, resulting in gene knockout. On the other hand, HR-mediated incorporation of exogenous donor DNA results in gene knockin. However, rapidly moving research field of genome editing has made this innovative technology extremely diverse and complex. In this talk, I provide basic and practical information on genome editing by showing a variety of examples from development to application of TALEN and CRISPR systems.

3-AS-01-2 Development of methods for reproducing disease-causing mutations using genome editing techniques in mice

Shuji Takada

Dept. of Systems BioMed., NCCHD

Genome editing technology enables us to produce knockout mice easily and quickly. Since this technology is more flexible than methods based on homologous recombination using ES cells, knockout mice can be produced for a gene which is surrounded by repetitive elements and designed at a single nucleotide level. In addition, production of knockout mice can be applied for the functional screening of mutations found in genomic analysis of patients by next generation sequencer to identify disease-causing mutations. Using genome editing technique, we have been constructing various application methods to reproduce disease-causing mutation identified in our institute in living mouse. In this talk, I will explain some of the methods we are using in my lab such as introduction of point mutation, production of KO mice for genes surrounded by repetitive elements, double KO mouse production of closely located genes and deletion of more than 25 kb genomic sequence. In addition, I will talk about generation of nested deletion mutants in mice to map disease-causing sequence.

3-AS-01-3 Cloning-free CRISPR/Cas system

Tomomi Aida

Lab. Mol. Neurosci., MRI, TMDU

Recent development of CRISPR/Cas-mediated genome editing technology has provided us the way to manipulate the genome of nearly any species freely and easily. Although this revolutionary technology enables the one-step production of knockout animals, several challenges still remain to be overcome such as transgene knock-in and specificity of editing in the animals. The use of Cas9 protein may be a key technology to these issues. We show cloning-free CRISPR/Cas system based on the direct nuclear delivery of Cas9 protein complex with chemically synthesized dual RNAs, which facilitates rapid one-step generation of transgene knock-in mice with high specificity. Our method expands the range of in vivo application of CRISPR/Cas technology.

3-AS-01-4 Single-step seamless CFTR gene correction in CF-iPSCs and their directed differentiation into cells responsible for CF pathophysiology

Shingo Suzuki[1,2], R. Geoffrey Sargent[2], Keisuke Chosa[2,4], Beate Illek[3], Horst Fischer[3], Alaleh Eamaeili-Shandiz[2], Tsuyoshi Shuto[4], Hirofumi Kai[4], Yuet. W Kan[1,5,6], Dieter. C Gruenert[2,6,7]

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Sequence-specific modification via homologous recombination (HR) in mammalian genomic DNA is an attractive, potentially therapeutic, strategy for inherited diseases. Sequence-specific endonucleases, such as the ZFNs, the TALENs and the CRISPR/Cas 9, significantly improves HR through introduction of double strand DNA breaks in the target genomic DNA. Combined with the induced pluripotent stem cells (iPSCs) technology these two technologies has advanced the potential of a personalized cell and gene therapy for genetic diseases. Here, we show single-step seamless CFTR gene correction in cystic fibrosis (CF) patient-derived iPSCs using small/short DNA fragments comprised of wild type donor DNA in conjunction with TALENs. Corrected iPSCs were clonally isolated by cyclic enrichment strategy that resulted in an ~100-fold enrichment of the corrected CF-iPSCs after 6 enrichment cycles. The corrected CF-iPSCs showed wild type airway epithelial cell cAMP-dependent Cl ion transport when differentiated into airway-like epithelial cells. The potential and challenges involved in achieving a comprehensive therapy for inherited diseases like CF, using gene-corrected iPSCs, will be discussed.

3-AS-01-5 Study on tissue-specific and developmental stage-specific roles of ubiqutin ligases using CRISPR/Cas9 system

Masayuki Kaneko

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Studies on endoplasmic reticulum (ER)-associated degradation (ERAD), in which unfolded proteins accumulated in the ER are selectively transported to the cytosol for degradation by the ubiquitin-proteasome system, have been focused on molecular mechanisms in yeast. In human, disruption of the ER quality control system causes various diseases. Furthermore, ER stress has become more important because it is also involved in cellular differentiation and tissue development. We have identified human 37 ubiquitin ligases with transmembrane domain, which are potentially involved in ERAD. As reason for so many genes in mammals compared with the yeast 2 ubiquitin ligases, they are assumed to have tissue-specific and/or developmental stage-specific roles. However, physiological roles of these 37 transmembrane ubiquitin ligases remain largely unkown. To address this issue, we used the next generation gene targeted editing tool, CRISPR/Cas9 system, to generate knock-out mise and stem cells. Here, we introduce current findings on the involvement of the ubiquitin ligases in novel biological functions.

3-AS-02-1 Drug discovery in big data era

Yasushi Okuno

Kyoto Univ. Grad. Sch. Med.

According to accelerating computing power such as super computers, the importance of "Simulation science" and "Big data science" is growing rapidly as modern sciences, second only to experimental science and theoretical science. In the life science field, along with the significant progress of equipment of measurement and observation and ICT technology in recent years, it is said to have entered the big data era. Especially, research and development of big data analysis techniques for personal genome is an urgent need. In the clinical medicine and epidemiology, big data is called as real world data, and the analysis of clinical real world data has attracted attention as a new approach which can reveal true fact that is happening in actual clinical site.For application of big data approaches to drug discovery, we have been developing the methods to predict compound–protein interactions, lead compound structures and clinical adverse drug reactions using artificial intelligence. In this presentation, we would like to discuss the potential of the big data approaches for pharmacology (especially systems pharmacology) and drug discovery.

3-AS-02-2 Particle simulations of epidermal growth factor response pathway

Koichi Takahashi

RIKEN QBiC

We have constructed a predictive model of epidermal growth factor (EGF) response pathways in mammalian cells at the molecular resolution usingK–computer. Cells take advantage of network structural,spatial, and temporal dimensions in design space to achieve and optimizetheir signaling functions. Building blocks where correlations betweennetwork, space and time are most evidently exhibited include clusteringand localization of receptor proteins, multi–site covalent modificationsof proteins such as double–phosphorylation of mitogen–activated proteinkinases (MAPK), and scaffold proteins which tether kinases andsubstrates in close proximity. An intriguing question is howcells respond to the signal differently. This phenomena called cellresponse heterogeneity is suggested to be relevant to some higherfunctions such as cell differentiation, development and immunology. Wefound that, among many possible factors, extrinsic noise (cell–to–cell variance in protein expression) can cause this heterogeneityin cellular response in a way that is consistent with experimentalobservations.

3-AS-02-3 Molecular dynamics simulation study on protein–small molecule interaction

Takefumi Yamashita

RCAST, Univ. Tokyo

The advancement of computational power and methodology has extended the range of application of molecular dynamics (MD) simulation. In particular, the MD simulation has been widely used to clarify the mechanism of protein function, which usually involves dynamic structural change not only of protein but also of hydrogen bond network of water molecules in the cavity (e.g., Yamashita and Voth, JACS 2012). In the context of pharmacology, protein–small molecule interaction is of special interest : the resulting molecular recognition in the cell is directly related to the drug efficacy and side effect minimization. Because an excellent property to represent the binding interaction between a protein and small molecule is the binding free energy, the accurate prediction of the binding free energy is critical to succeeding in the computer–assisted drug design. Recently, we tried to improve the prediction accuracy by introducing the MD simulation–based binding free energy calculation method with the huge computational power of "K", which is one of the fastest computers in the world (e.g., Yamashita et al., Chem. Pharm. Bull. 2015). We discuss the possibility that the accurate MD simulation makes the drug development more efficient and rational.

3-AS-02-4 Theory of Dynamical Network Biomarkers and Mibyou and Preemptive Medicine

Kazuyuki Aihara

IIS, Univ. Tokyo

In this talk, I will review our recent research of dynamical network biomarkers theory and its possible application to mibyou and preemptive medicine. Dynamical network biomarkers (DNB) are a new concept of biomarkers that detect a predisease or mibyou state rather than a disease state. DNB is derived mathematically on the basis of bifurcation theory, and can be used to identify early warning signals for an imminent bifurcation point from a healthy state to a disease state, namely a predisease state. Due to this diagnosis of the mibyou state, preemptive treatment can be started early enough beforeemergence of disease symptoms. Further, I will discuss possible pharmacological research for such preemptive medicine from the viewpoint of DNB.

3-JPS-1 NMDA receptor function and human disease

Stephen Traynelis

Dept. Pharmacol., Emory Univ. Sch. Med.

NMDA receptors are ligand-gated channels that mediate a slow, Ca^{2+}-permeable component of excitatory synaptic transmission in the central nervous system. The receptors are tetrameric assemblies typically comprised of two GluN1 and two GluN2 subunits, encoded by a family of five genes (*GRIN 1*, *GRIN2A*, *GRIN2B*, *GRIN2C*, *GRIN2D*). De novo and inherited mutations within this receptor family identified in patients with neurological disease but absent from the healthy population are most commonly associated with epilepsy. The *GRIN2A* gene harbors more of these rare variants than other *GRIN* genes, and thus has been considered a locus for childhood epilepsy. Despite the identification of hundreds of NMDA receptor mutations, there are less than a dozen variants for which functional data exist. We have sought to advance our understanding of the role of these mutations in neurological disease by comprehensively obtaining functional data on all variants currently in the literature as well as new cases identified by our collaborators. These studies have revealed patient populations that may be amenable to therapeutic treatment. These studies have also provided new information about how the NMDA receptor works by highlighting key residues involved in channel gating.

3-JPS-2 Glutamate receptors, synapse organizers and intellectual disability

Masayoshi Mishina

Brain Sci. Lab., Res. Org. of Sci. Tech., Ritsumeikan Univ.

Acquiring new knowledge is one of the most important functions of our brain. Disturbance of learning and memory affects our cognitive capabilities and thus our quality of life, such as intellectual disabilities (ID) and Alzheimer disease. We have been studying the molecular basis of learning and memory focusing on the glutamate receptor (GluR), the key molecule of information transmission between neurons. We showed that the NMDA receptor GluRε1/GluN2A determines the thresholds of hippocampal synaptic plasticity LTP and contextual learning. Cerebellar synaptic plasticity LTD and optokinetic response adaptation are facilitated in mutant mice lacking GluRδ2-interacting protein Delphilin. We also found that the NMDA receptor GluRε2/GluN2B is essential for sensory map formation in the brain and GluRδ2 regulates cerebellar synapse formation by trans-synaptic interaction with presynaptic neurexins through Cbln1. Synapse formation of cortical neurons is mediated by IL1-receptor accessory protein-like 1 (IL1RAPL1), responsible for nonsyndromic ID and autism. Impairments of synapse formation and maturation are implicated in the pathogenesis of mental disorders. Thus, we are beginning to uncover the molecular basis of brain functions and mind.

3-JPS-3 Cognitive improvement through CaMKII activation by the hippocampal T-type calcium channels

Kohji Fukunaga

Dept. Pharmacol. Tohoku Univ. Grad. Sch. Pharm. Sci.

We here introduce a novel Alzheimer disease (AD) therapeutics candidate, which has potential to enhance of CaMKII activity in the hippocampus and prefrontal cortex. We first define the mechanism underlying cognitive enhancement of a novel AD drug candidate, ST101 which stimulates T-type calcium channel in mouse cortical slices (Moriguchi et al., J Neurochem 2012 ; 121 : 44-53). ST101 finished the Phase II trial in U.S.A. (J Alz Dis 2015 ; 48 : 473). We then successfully synthesized more potent spiroimidazopyridine derivatives (SAK3), which markedly enhanced CaMKII activity, thereby promoting LTP induction and maintenance in the hippocampus. Interestingly, SAK3 stimulated T-type calcium channels in neuro2A cells transfected with CACNA1G (Cav3.1) and CACNA1I (Cav3.3), but not CACNA1H (Cav3.2). The CaMKII activation is associated with increases in ACh and glutamate release in the hippocampus. The enhancements of ACh and glutamate release were completely blocked by pre-administration of T-type calcium channel blocker NNC 55-0396. Furthermore, Cav3.1 channel was highly expressed in the terminal of ACh neurons in the hippocampus, suggesting that SAK3 improves memory deficits via stimulation of T-type calcium channels.

3-JPS-4 Tea polyphenols inhibit rat osteoclast formation and differentiation

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Regulation of osteoclast activity is essential in the treatment of bone disease. Polyphenols in green tea, particularly epigallocatechin-3-gallate (EGCG), inhibit MMPs expression and activity. However, the effects of the black tea polyphenol, theaflavin-3,3'-digallate (TFDG), on osteoclast and MMP activity are unknown. Therefore, we examined whether TFDG and EGCG affect MMP activity and osteoclast formation and differentiation in vitro. TFDG or EGCG (10 and 100 μM) was added to cultures of rat osteoclast precursors cells and mature osteoclasts. Numbers of multinucleated osteoclasts and actin rings decreased in polyphenol-treated cultures relative to control cultures. MMP-2 and MMP-9 activities were lower in TFDG- and EGCG-treated rat osteoclast precursor cells than in control cultures. MMP-9 mRNA levels declined significantly in TFDG-treated osteoclasts in comparison to control osteoclasts. TFDG and EGCG inhibited the formation and differentiation of osteoclasts via inhibition of MMPs. TFDG may suppress actin ring formation more effectively than EGCG. Thus, TFDG and EGCG may be suitable agents for the treatment of bone resorption diseases.

3-JPS-5 Nicotine- and tar-free cigarette smoke extract induces cell damage through reactive oxygen species newly generated by PKC-dependent activation of NADPH oxidase

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We examined cytotoxic effects of nicotine/tar-free cigarette smoke extract (CSE) on C6 glioma cells. The CSE induced plasma membrane damage and apoptosis. The cytotoxic activities decayed with half-life of approximately 2 h at 37℃, and were abolished by N-acetyl-L-cysteine and reduced glutathione. The membrane damage determined by LDH leakage and propidium iodide uptake was prevented by catalase and edaravone (a scavenger of $^{\cdot}OH$) but not by superoxide dismutase, indicating involvement of $^{\cdot}OH$. In contrast, the apoptosis determined by MTS assay and DNA fragmentation was resistant to edaravone, but induced by either authentic H_2O_2 or O_2^- generated by xanthine/xanthine oxidase system, indicating involvement of H_2O_2. Diphenyleneiodonium (NOX inhibitor) and bisindolylmaleimide I abolished membrane damage, whereas they partially inhibited apoptosis. These results demonstrate that 1) stable components in the CSE activate PKC, which stimulates NOX to generate reactive oxygen species (ROS), causing membrane damage and apoptosis ; 2) different ROS are responsible for membrane damage and apoptosis ; 3) part of the apoptosis is caused by oxidants generated independently of PKC and NOX.

2-CS-1 Mode of SGLT inhibition by an SGLT2 inhibitor, canagliflozin and implication in renal and small intestinal effects

Chiaki Kuriyama

Research Unit C, Sohyaku. Innovative Research Division

SGLT2 inhibitors reduce blood glucose levels through increasing urinary glucose excretion. SGLT2 is expressed in the proximal tubules in the kidney, whereas SGLT1 is located also in the small intestine. Both SGLTs localize in the luminal but not the basolateral side of epithelial cells. We analyzed the sidedness of inhibition of SGLTs by canagliflozin (Cana) to clarify exact PK/PD profiles. Cana inhibited SGLT2 from the extracellular side but not from the intracellular side, and the inhibition of glucose transport was competitive and depended on the extracellular Cana concentration. SGLT1 was also inhibited by Cana at higher concentration range in a similar manner. PK analysis revealed that the plasma level of Cana is sufficient to inhibit SGLT2 but not SGLT1, whereas intestinal luminal concentration is expected to be high enough to inhibit SGLT1 after oral treatment. In OGTT in rodents, Cana improved blood glucose excursion, probably by inhibiting intestinal SGLT1 as well as renal SGLT2. A recent clinical study of Cana also found the delayed intestinal glucose absorption accompanied by increased plasma GLP-1 levels. These results indicate that oral Cana transiently inhibits intestinal SGLT1 in addition to renal SGLT2.

2-CS-2 SGLT2 Inhibitor (Luseogliflozin): A New Mechanism for Treating Type 2 Diabetes Mellitus and Therapeutic Potential to Prevent the Progression of Diabetic Complications

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SGLT2 inhibitor has the unique mechanism of action on blood glucose lowering effect.
In Zucker fatty rats, luseogliflozin as SGLT2 inhibitor increased urinary glucose excretions and lowered the increase in blood glucose levels on oral glucose tolerance test. Furthermore, luseogliflozin suppressed the increase in plasma insulin levels after glucose loading.
In Japanese patients with type 2 diabetes, luseogliflozin increased urinary glucose excretion and lowered the mean 24 hours blood glucose levels measured by continuous glucose monitoring. The proportion of time during 24 hours with glucose levels of 70-180 mg/dL was increased without hypoglycemia. The decrease in blood glucose levels was accompanied by reduction in serum insulin levels throughout the day.
These results suggest that luseogliflozin could lead to favorable improvement in glycemic control independent of insulin secretion in diabetic animals and also type 2 diabetic patients. Moreover, luseogliflozin prevented the progression of renal injury in animal models of diabetic nephropathy. Further investigation of SGLT2 inhibitors on diabetic complications would be expected to be innovative therapeutic options.

2-CS-3 Pharmacological analysis of SGLT2 inhibitor (tofogliflozin) using in vivo glucose clamp and titration protocols in rats and cynomolgus monkeys

Masanori Fukazawa, Takumi Nagata, Masayuki Suzuki, Yoshiyuki Suzuki, Yoshiki Kawabe

Fuji Gotemba Research Labs. Chugai Pharm.

Using the highly specific SGLT2 inhibitor tofogliflozin (TOFO) and the SGLT1 and 2 (SGLT1/2) inhibitor phlorizin (PHZ), we evaluated : 1) the ratio of contribution of SGLT2 vs. SGLT1 to renal glucose reabsorption (RGR),2) the risk of hypoglycemia from urinary glucose excretion (UGE), and 3) the effects on TmG (transport maximum for glucose) and splay of the glucose titration curve. Under hyperglycemic conditions in rats, TOFO and PHZ achieved>50% inhibition of RGR when plasma levels were sufficient to inhibit SGLT2 completely and SGLT1 to different degrees. Under hypoglycemic conditions, RGR was reduced by 20-50% with PHZ and by 1%-5% with TOFO. Under normoglycemic conditions, endogenous glucose production (EGP) increased to compensate for TOFO-induced UGE, resulting in normal plasma glucose (PG) levels, but higher UGE and a greater EGP induced by PHZ resulted in lower minimum PG. Fitting of the titration curve in cynomolgus monkeys showed that TOHO and PHZ extend the splay without affecting TmG. These results suggest that the contribution of SGLT1 to RGR is greater under lower glycemic conditions, and that SGLT2-selective inhibitors pose a lower risk of hypoglycemia than SGLT1/2 inhibitors.

2-CS-4 Visualization of mechanism of action, and a further therapeutic potential of ipragliflozin, a selective SGLT2 Inhibitor

Toshiyuki Takasu, Shoji Takakura, Kaori Hamada, Minori Saitoh

Drug Discovery Research, Astellas Pharma Inc.

In this session, the presenter will introduce the recent findings of ipragliflozin (ipra), the first C-glycoside SGLT2-selective inhibitor synthesized in cooperation with Kotobuki Pharmaceuticals Co. Ltd. Using positron emission tomography, the mechanism of action of ipra in rat kidney was visualized directly and dynamically. In order to investigate pharmacological potential by inhibiting SGLT2, we examined the effect of ipra on body composition of diet-induced obese (DIO) rats. Further, effect on the development of non-alcoholic fatty liver disease (NAFLD) was studied in choline-deficient L-amino acid-defined (CDAA) diet rats. As a result, it was revealed that ipra reduced visceral and subcutaneous fat masses but not lean mass or bone mineral content in DIO rats, which was accompanied with enhanced lipolysis and fatty acid oxidation. In CDAA diet-fed rats, ipra prevented both hepatic TG accumulation and large lipid droplet formation, and exerted a prophylactic effect on liver fibrosis.
These findings suggest a variety of pharmacological action of SGLT2 inhibitors caused by enhanced urinary glucose excretion.

3-CS-1 Translating novel therapeutics for neurodegenerative disorders

Masanori Hizue

Drug Safety R&D, Pfizer Inc.

As the world's population continues to age, neurodegenerative disorders such as Alzheimer's and Parkinson's diseases represent significant unmet medical needs that require transformational therapies. Without effective therapies, these disorders will present significant societal, economic, and ethical challenges. Significant effort within the pharmaceutical industry is focusing on delivering efficacious therapies that could potentially modify the course of disease progression as well as provide novel symptomatic treatment paradigms. By utilizing a deep understanding of disease pathogenesis our success at delivering innovative medicines should improve. The application of human genetics informs target discovery, validation and identification of pathways involved in disease pathogenesis and focuses opportunities to utilize a precision medicine based approach. We are currently focusing on novel mechanisms rooted in human genetics to discover small molecule disease modifying therapies for Alzheimer's disease in addition to focusing on agents that target specific domains associated with neuropsychiatric disorders. In this presentation, two case studies utilizing these approaches will be discussed.

3-CS-2 A strategy to improve the success rate of CNS drug development: TAK-063, a novel PDE10A inhibitor, as a case study

Haruhide Kimura

CNS Drug Discovery Unit, Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited

Principal mechanisms of current medications for psychiatric diseases were primarily discovered by serendipity. The success rate for central nervous system (CNS) drug development is still low for several reasons, including a lack of knowledge of disease pathophysiology and poor predictive validity of animal models. This presentation describes a strategy of CNS drug development using a phosphodiesterase 10A (PDE10A) inhibitor, TAK-063, as an example. By careful consideration of the biology of the target and detailed biochemical studies, we hypothesized that off-rates of PDE10A inhibitors characterize their pharmacological profile via the differential activation of the direct and indirect pathways in the stratum. Based on this hypothesis, we discovered the PDE10A selective inhibitor TAK-063 with balanced activation of these pathways via a faster off-rate profile. Several biomarkers, including PDE10A occupancy by positron emission tomography, electroencephalogram, and pharmacological magnetic resonance imaging, were used to bridge preclinical and clinical studies. Original strategies to find differentiated drugs and integration of translational studies will be a key to improving the success rate for CNS drug development.

3-CS-3 How to improve success rates of the compounds acting on CNS using biomarkers

Seishi Katsumata

Dis. Res. Lab. I, Dept. of Biology & Pharmacology ONO Pharmaceutical Co., Ltd

Regardless of the huge R&D investments, less than 10% of the CNS drug candidates have been approved in US. The low success rate is attributed to lack of efficacy in the late phases of clinical trial. Genetic, blood, CSF, electrophysiological, and imaging biomarkers are expected to contribute to identifying right patients, selecting doses and deducing efficacy. Among them, PET is often used for go/no go decision making.
In the phase 3 trial of anti Aβ antibody for Alzheimer's disease, Aβ PET is used for identifying the patients with Aβ deposits in the brain. In the phase 1 trial of TSPO antagonist, ONO-2952, relationship between plasma concentration and TSPO occupancy was determined to select optimal doses in the phase 2 trial for IBS. ONO-2952 could improve visceral pain and bowel habits in female diarrhea predominant IBS patients with the predicted doses. Lack of efficacy of aprepitant in several phase3 trials for depression with the doses in which it occupies more than 80% of the NK1 receptor in the brain suggests that NK1 receptor could not be appropriate target for antidepressant.
These challenges indicate that biomarkers can contribute to improving success rates of CNS drug candidates and early decision making for further development.

1-YS-1 Comprehensive cell analysis of whole organ/body for the organism-level systems biology

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The multicellular organism is compose of various types of cells inside, where they connect with each other and work in a coordinated manner. The mechanism of an organism-level biological function, such as the sleep-wake behavioral rhythm, is implemented in the multicellular circuitry or the multicellular system. In this talk, we will introduce a comprehensive cell and cell circuit analysis pipeline, termed CUBIC (Clear, Unobstructed Brain/Body Imaging Cocktails and Computational analysis). This includes an efficient and reproducible whole organ and body clearing method, a rapid imaging with light-sheet fluorescence microscopy and computational image informatics, and supports the realization of *the organinsm-level systems biology*. This technology thus can be applicable to the wide range of life science and medical researches, and will facilitate our system-level understanding of the multicellular organism. References : Susaki et al. Cell 157 : 726-739, 2014Tainaka et al. Cell 159 : 911-924, 2014Susaki et al. Nature Protocols 10, 1709-1727, 2015.

1-YS-2 Neural Circuit Tracing with Glycoprotein-Deleted Rabies Viruses

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The processing of neural information in neural circuits plays a key role in brain functions. To gain mechanistic insights about brain functions and to treat diseases of the nervous system, it is crucial to understand what the neural circuits are computing and how they achieve these computations. However, studying a specific neural circuit is extremely difficult because most nervous system structures contain many types of neurons with intertwined axons and dendrites. Here I will describe rabies virus-based system that makes it possible to resolve connectivity with high resolution, to correlate connectivity with function, and to manipulate the activity of defined circuit components. Rabies viruses have been used for anatomical studies because they spread transsynaptically in the retrograde direction and only between connected neurons Our system is based on rabies viruses whose glycoprotein gene has been deleted from the genome, making it possible to control the transsynaptic spread of the virus and to select specific neurons or neuron types for primary infection. This also allows circuits of specific neurons to be more directly linked to function for physiological and behavioral studies.

1-YS-3 Optogenetic fMRI for the study of BOLD signal induction by astrocytes

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Functional magnetic resonance imaging (fMRI) to localize brain area responding to drug challenges is called pharmacological MRI (phMRI). fMRI measures blood oxygenation level dependent (BOLD) signal, which has been used as a surrogate marker of neuronal activities. Astrocyte, another major component in the brain, has been assigned a subsidiary role in BOLD signal generation ; responding to neuronal activities, astrocytes dilate blood vessel diameter, resulting in BOLD signal increase by changing oxy- and deoxy-hemoglobin ratio. We hypothesized that astrocytes might also consume oxygen and induce BOLD signal, which has been difficult to test because conventional methods stimulate both neurons and astrocytes. To this end, we combined recent technological breakthroughs : optogenetic activation of astrocytes in vivo (Cell Rep. 2 : 397-406, 2012) and mouse specific MRI receiver (CryoProbe). We first prepared a transgenic mouse whose astrocytes express a light-sensitive cation channel, channel rhodopsin-2. CryoProbe enabled us to perform fMRI from a small brain of a mouse. We observed BOLD signal upon the optogenetic activation of astrocytes without apparent modulation of neuronal activities. Our results may suggest astrocytes as a new pharmacological target in phMRI.

1-YS-4 Visualization of Ca^{2+} dynamics within the endoplasmic reticulum for the investigation of neuronal and astrocytic functions

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Ca^{2+} is a key messenger that regulates neuronal and astrocytic functions in the brain. Although the endoplasmic reticulum (ER) plays indispensable roles as a source and sink of Ca^{2+}, technical difficulties have impeded analysis of Ca^{2+} dynamics within the ER. In this study, we have used a genetically encoded ER Ca^{2+} indicator, G-CEPIA1*er* to visualize Ca^{2+} dynamics within ER. We found that Ca^{2+}-mobilizing synaptic inputs locally decreased the ER Ca^{2+} concentration, followed by Ca^{2+} replenishment by intraluminal Ca^{2+} diffusion throughout the ER of dendrites and spines. Furthermore, Ca^{2+} spike-inducing synaptic inputs cumulatively increased the ER's Ca^{2+} content. Thus our study indicates that the ER functions both as a tunnel to redistribute stored Ca^{2+} and as a leaky integrator of synaptic inputs in neurons. We also found ER Ca^{2+} dynamics in astrocytes induced by behavior and drug treatment. Collectively, these ER Ca^{2+} visualization studies provide important new insights to ER Ca^{2+} dynamics in neurons and astrocytes.

2-YS-1 Pharmacogenetics to study synapse competition

Ryuta Koyama

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The formation of mature neural circuits requires selective pruning of inappropriate synapses and strengthening of appropriate synaptic connections, i.e., synapse competition. A longstanding question in neurobiology is what determines which synapses will be eliminated? While the role of spontaneous and experience-driven synaptic activity in developmental synaptic pruning is well established, surprisingly little is known about the molecules and mechanisms that link neural activity with the physical elimination of specific synapses. In order to dissect the mechanisms that drive the elimination of specific CNS synapses, a reliable in vitro model is necessary. In this talk, I will introduce a novel retinogeniculate co-culture system in which the activity of axons from retinal ganglion cells can be modulated by pharmacogenetics : we used the DREADD (Designer Receptors Exclusively Activated by Designer Drugs)-system, in which the activation of G-protein coupled receptors (Gq, Gi, and Gs) engineered from muscarinic receptors can be precisely controlled by a ligand named clozapine N-oxide (CNO) which is otherwise pharmacologically inert.

2-YS-2 *In vivo* analysis of reward signal based on flex system: mode of action in D1R MSNs

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It is well known that dopamine (DA) is necessary for motor function, motivation, working memory and reward. The principal target of DA is medium spiny neurons (MSNs), which are a special type of GABAergic inhibitory cell that represents 95% of the neurons within the striatum, including the nucleus accumbens (NAc). There is a distinct class of spatially intermixed MSNs that express DA type 1 or 2 receptors (D1R-MSNs or D2R-MSNs, respectively). D1R is coupled to adenylate cyclase through G_{olf} to activate protein kinase A (PKA), whereas D2R inhibits adenylate cyclase through G_i. Although PKA has been implicated in reward signals downstream of D1Rs by pharmacological approaches, there is no direct evidence indicating that PKA in D1R-MSNs regulates neuronal excitability and reward-related behaviors. We established the system in which wild-type PKA (wtPKA) or constitutively active mutant PKA (caPKA) was expressed in the NAc under the control of specific promoters for D1R-MSNs using adeno-associated virus (AAV)-mediated conditional transgenic techniques. In this symposium, I will discuss *in vivo* analysis of reward signal based on flex system to elucidate mode of action in D1R MSNs.

2-YS-3 Multi-unit recording with microgenetic manipulation for studying hippocampal information processing

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Firing patterns of individual neurons are determined by the interaction of synaptic inputs with cellular mechanisms. To investigate the cellular mechanism mediating a specific firing pattern, interfering with the cellular machinery of interest under intact input activity is crucial. To achieve this, we devised a virus-mediated approach to perform extracellular unit recording from genetically manipulated neurons in the intact brain environment, and examined a role of synaptic plasticity for hippocampal firing activity. We showed that synaptic plasticity dependent on the GluR1 subunit of AMPA receptor mediates two dynamic changes in neuronal firing in the hippocampal CA1 area during novel experiences : (i) the establishment of phase-locked firing to slow gamma oscillations, which is thought to originate from the CA3 area, and (ii) the rapid formation of the spatial firing pattern of place cells. The results suggest a series of events potentially underlying the acquisition of new spatial information : slow gamma oscillations induce the two GluR1-dependent changes of CA1 neuronal firing, which in turn determine information flow in the hippocampal-entorhinal system.

2-YS-4 FAST: a new block-*f*ace *s*erial microscopy *t*omography for high-speed imaging and an unbiased comparative analysis

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Recent remarkable progress in optical microscopy has facilitated whole brain imaging to systemically understand anatomical and functional brain networks. However, whole-brain imaging at a resolution sufficient to discriminate all of the cells in the brain is still challenging due to trade-offs between imaging speed and spatial resolution. Here we have developed a new automated imaging system, named FAST (block-*f*ace *s*erial microscopy *t*omography), that consists of a confocal microscope and a built-in tissue slicer system. FAST system provides whole brain images at subcellular resolution with unprecedented speed. In combination with fluorescence labeling methods, FAST system enables the visualization of neural activities and neural circuits in the whole brain. In addition, we achieved unbiased and quantitative comparisons of the whole brain images. The quantitative comparison detected cell loss and neural activities induced by various stressors. Thus, FAST system can provide not only detailed brain mapping, but also comprehensive information about structural and functional changes in the brain, which contribute to a solid understanding of brain systems.

1-PMS-1 Immunology cures diverse rheumatic diseases: a perspective on immunopharmacology

Masaru Ishii

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Drugs targeting the immune system have been widely exploited in the treatment of inflammatory, allergic and autoimmune disorders during the second half of the 20th century. The recent advances in immunopharmacological research made available new classes of clinically relevant drugs, comprising protein kinase inhibitors and biologics, such as monoclonal antibodies that selectively modulate the immune response not only in cancer and autoimmunity but also in a number of additional human pathologies. The newly formed ImmuPhar is the Immunopharmacology Section of the International Union of Basic and Clinical Pharmacology, providing a unique international expert-lead platform aiming to dissect and promote the growing understanding of immune system as well as to challenge the identification and validation of drug targets and lead candidates for the treatment of many forms of debilitating diseases including, among others, cancer, allergies, autoimmune and metabolic diseases. In this symposium I will outline the current movement on immunopharmacology in Japan as well as in the world, focusing on the activity of ImmuPhar, and discuss future perspectives in this promising field.

1-PMS-2 Inflammatory mediators that regulate immune cells and therapeutic targets

Masataka Majima

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Inflammation is a set of complex biological responses to harmful stimuli such as pathogens, damaged cells, and irritants. Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators, and its purpose is to eliminate the initial cause of cell injury, to clear out tissues damaged from the original insults, and to initiate tissue repair. We had shown that authentic inflammatory mediators, arachidonic acid metabolites including prostaglandins (PGs) and leukotrienes (LTs) regulated immune cells and became good therapeutic targets. A PGE receptor, EP signaling enhanced chronic inflammation via inducing isoforms of vascular endothelial growth factors that regulate angiogenesis and lymphangiogenesis. Further, it also facilitated tumor lymph node metastasis via formation of premetastatic niche affecting dendritic cells and regulatory T cells. A LT receptor, BLT1 signaling exhibited the chemotactic activity on mast cells to form the proangiogenic microenvironment. These indicate that the field of arachidonic acid metabolites is quite rich with therapeutic candidates that modulate immune cell functions.

1-PMS-3 Significance of Clinical Pharmacology in Development of Drugs for Autoimmune Diseases.

Shinichi Kawai

Div. Rheumatol., Dept. Intern Med., Toho Univ. Sch. Med.

Clinical pharmacology is an academic field of investigating clinical use of drugs. It is tightly linked to the basic pharmacology, with special focus on the application of pharmacological theory to the actual clinical therapeutics. Clinical pharmacology includes wide spectra from clinical development of drugs against new target molecules, to the practical information of how to use drugs. The etiology of autoimmune diseases remains unclear at present, but advances have been made in the elucidation of its pathophysiology based on progress in molecular biology and related fields. As a consequence, many attempts have been made to develop new methods of treatment. Especially, recent treatment of rheumatoid arthritis has been advanced dramatically.In this presentation, I will introduce the current drug development for autoimmune diseases. In addition, I will also introduce our recent clinical studies conducted to elucidate the ethnic differences in pharmacokinetics of several drugs among the east Asian populations and Caucasians.

1-PMS-4 A novel direction of human immunology research and its relevance to drug discovery

Kazuhiko Yamamoto

Dept. Allergy and Rheumatology, The Univ. Tokyo, Sch. Med

Although basic concept of immunology has been greatly progressed using mouse system, understanding human immune system remains unsatisfactory. One of the advantages of mice is knockout technology, which clearly indicates targeted gene is the cause of phenomena. In human, it is impossible to utilize such technology. However, gene polymorphisms are inherited prior disease. Therefore, disease risk polymorphisms indicate the causality of disease.Recent genome wide association studies (GWAS) have uncovered numerous risk genes. We conducted a trans-ethnic GWAS meta-analysis for rheumatoid arthritis (RA) through international collaboration and identified 101 RA risk loci. A systematic evaluation of the overlap between RA risk genes and the target genes of approved drugs suggest that disease risk genes are promising resources for drug discovery. Forty four out of 100 non-HLA SNPs were found in cis-acting expression quantitative trait loci (cis-eQTL). This indicates that majority of disease-causing variants affect the expression of genes. Using these findings, we believe that we can construct pathway interactions of genes and cells, which will unravels complex responses of immune related genes and cells and will give novel insights into human immune system.

2-PPS-1 Optogenetic control of descending noradrenergic system and in vivo analysis of its antinociceptive action

Hidemasa Furue

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Locus coeruleus (LC) neurons in the brain stem send noradrenergic projections throughout the neuroaxis and are implicated in the control of many homeostatic functions such as arousal, cardio-respiratory control. In addition, the LC is also a major source of noradrenergic projections to the spinal superficial dorsal horn which play an important role in pain modulation. Clinically alpha 2-adrenoceptor agonists are widely used as sedative agents, and LC is also principal site for their sedative action. We have examined how descending noradrenergic neurons inhibit spinal nociceptive transmission by using in vivo recordings of spinal nociceptive synaptic transmission and optogenetic control of LC neurons. Optoactivation of the LC neurons expressing ChR2 elicited a barrage of spinal inhibitory GABAergic synaptic responses. This activation was mediated by alpha 1 receptors expressed in spinal GABAergic neurons. Systemic administration of alpha 2-agonist also enhanced the descending noradrenergic control of spinal GABAergic transmission at low doses that produce minimal sedative action. This novel analgesic mechanism may provide new targets for intervention allowing analgesic actions to be dissociated from unwanted sedation.

2-PPS-2 Role of orexin neurons on pain sensation

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Orexin is a neuropeptide synthesized small number of neurons located in the hypothalamus. Recent study revealed that sleep disorder Narcolepsy is caused by specific loss of orexin neurons. This fact suggests that orexin neurons have an important role in the regulation of sleep/wakefulness. However, narcolepsy patients show not only abnormality of sleep/wakefulness but also abnormality of metabolism. Additionally, narcolepsy patients showed increase in chronic pain suggesting role in pain sensation. Here we generated Narcolepsy model mice which orexin neurons are specifically ablated at desired timing. Orexin ablated mice showed decreased in pain threshold means sensitive to pain. Conversely, activity of orexin neurons are activated by using pharmacogenetics. Activation of orexin neurons increased pain threshold indicates that mice showed analgesia. Finally, we measured the activity of orexin neurons in free behaving mice by using fiber photometry. Calcium probe, GCaMP6, was exclusively expressed in orexin neurons. Mice were applied by mechanical pain and heat pain. The activity of orexin neurons was increased by applying mechanical or heat pain. Taken together, we revealed that the activity of orexin neurons have an important role in the sensing pain.

2-PPS-3 New approach for elucidating neuropathic allodynia using optogenetics

Makoto Tsuda

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A cardinal symptom of neuropathic pain is characterized by tactile allodynia (pain hypersensitivity evoked by innocuous mechanical stimuli). Effective therapy for this pain is lacking, and the mechanisms are unclear. Since myelinated primary afferent Aβ-fibers transmit innocuous mechanical information from the skin to the spinal dorsal horn (SDH), it has been hypothesized that pathological altered signaling from Aβ-fibers might be implicated in tactile allodynia. However, there is no tool to selectively stimulate Aβ-fibers *in vivo*, and thus whether stimulation of Aβ-fibers causes pain after PNI remains unknown. In this talk, I will show our recent findings using optogenetics. After PNI, blue light stimulation of the plantar skin of transgenic rats (ChR2V) that had expressed channelrhodopsin-2 mainly in Aβ-fibers among primary afferent populations, exhibited pain-like withdrawal responses. Electrophysiological studies revealed the existence of excitatory inputs from Aβ-fibers to lamina I SDH neurons in PNI-ChR2V rats, implying a conversion of Aβ-fiber-mediated innocuous information to pain. Thus, this might provide a new approach for investigating the mechanisms underlying neuropathic allodynia and evaluating the analgesic effects of drugs.

2-PPS-4 Analysis of "pain-activated network" and "pain-ON cells" for identification of the mechanism of chronic pain

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Chronic pain, especially, neuropathic pain, often has a negative effect on the patient's quality of life, can act as a stressor and increases the incidence of anxiety and depression. However, the molecular mechanism of such refractory pain is not fully understood. An optogenetic approach and a designer receptor exclusively activated by designer drug (DREADD) system have emerged as powerful tools for studying the "pain-activated network". As well as the neuronal network analysis, the "pain-ON cell" sorting and its multi-Omics profiling are useful for identifying the molecular mechanism of chronic pain. Additionally, more recent advances using highly reproducible and sensitive detection methods have improved our ability to document cellular phenotypic variation of "pain ON-cells". In this symposium, I will introduce our recent multi-analysis for the "pain-activated network" and "pain-On cells".

2-PTS-1 Progress in Gas Phamacology

Akio Matsumoto

Dept. Pharmacol., Chiba Univ. Grad. Sch. Med.

Although it has been known that a various gases are produced particularly in the gut by microbes, an endogenous gas production such as NO and CO in mammalian cells has established entirely new principle for intercellular signaling since gaseous molecule can easily travel through membrane and migrate into adjacent cells. These small molecules activate mainly cGMP pathways ; furthermore, they utilize thiol-reactivity to exert their biological functions in all classes of signaling pathways. Biochemical modifications on thiol by these molecules usually compete with O_2. Thus, gas signaling has to consider O_2 concentration at the site of action. Much attention has been paid to the chemical properties of thiol-modification to segregate the mode of action by each molecule. Recent findings on poly-sulfur modification have provided variable clues on the accumulated knowledge. Although it has not been clarified the molecular mechanism to exert the biological action, molecular hydrogen is coming into the field. Besides the anti-oxidative activity to eliminate $OH^{\cdot}$, molecular hydrogen potentiates β-receptor signal and transfer the activity. This session will provide basic concepts of thiol-based modifications with gaseous molecules and their clinical implications from the point of view in gas pharmacology.

2-PTS-2 Molecular mechanism of ER dysfunction triggered by nitric oxide

Takashi Uehara

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Treatment with high concentration or prolonged exposure of nitric oxide (NO) results in neuronal cell death or neurodegenerative disorders such as Parkinson's or Alzheimer's disease. We have demonstrated that NO induces S-nitrosylation of protein disulfide isomerase (PDI) which specifically exists in the lumen of endoplasmic reticulum (ER). The protein S-nitrosylation contributes to the accumulation of immature unfolded proteins due to the inactivation of PDI enzymatic activity via that reaction. In general, the specific signals in response to ER stress are activated via the sensors including IRE1, PERK, and ATF6, located in the ER membrane. We are attempting to resolve how the sensors are activated by NO in neuronal cells. Using a specific detection system for unfolded protein response (UPR) signal, we recently found that IRE1-XBP1 branch is preferentially inactivated by treatment with NO donors. Our findings reveal that nitrosative stress leads to dysfunctional UPR, thus contributing to neuronal apoptosis/neurodegenerative diseases.

2-PTS-3 Increase in cardiac risk by electrophile-mediated activation of dynamin-related protein 1

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Covalent modification of GTP-binding protein by endogenous electrophiles, such as 8-nitro-guanoshine 3',5'-monophosphate (8-nitro-cGMP), promotes myocardial early senescence. Exogenous treatment with NaHS improved heart failure by eliminating 8-nitro-cGMP accumulation. However, using a novel techniques to measure intracellular reactive sulfur species, we found that NaHS per se hardly eliminates electrophiles in vitro, and formation of more nucleophilic sulfur species, such as Cys persulfide/polysulfide in proteins, predominatly eliminates endogenous electrophilies in the heart. We also revealed that hypoxic stress or exogenous treatment with an electrophile, such as methylmercury (MeHg), induces mitochondrial hyper-fission via electrophilic modification of rodent dynamin-related protein 1 (Drp 1). Polysulfide detection assay revealed that endogenous Drp1 forms Cys persulfide in rat cardiomyocytes, and persulfide level is dramatically reduced by MeHg exposure. Treatment with NaHS for 24 hrs completely suppresses MeHg-induced activation of Drp1 and mechanical stress-induced cardiac injury. These results strongly suggest that S-polythiolation of Drp1 underlies suppression of eletrophile-mediated cardiac vulnerability to hemodynamic overload.

2-PTS-4 Capture of reactive sulfur species by environmental electrophiles

Yoshito Kumagai

Fac. Med. Univ. Tsukuba

Environmental electrophiles covalently modify protein thiols, resulting in protein adducts formation. For example, methylmercury (MeHg) activates some redox-signal transduction pathways through covalent modification of sensor proteins at nontoxic concentrations. It is therefore recognized that activation of the signaling pathways associated with cellular protection causes elevation of the threshold for environmental electrophile-mediated cell damage. We isolated an unknown metabolite of MeHg from SH-SY5Y cells exposed to MeHg and from the liver of rats given MeHg, which we identified as $(MeHg)_2S$. Our recent findings indicate that $(MeHg)_2S$ is produced as a result of the capture by MeHg of a mobilized sulfur atom from endogenous reactive sulfur species (RSS) such as GSH persulfide (GSSH), its polysulfide (GSSSG) and even protein-bound RSS. Because RSS have high nucleophilicity and antioxidant capability, substantial $(MeHg)_2S$ formation is associated with a reduction in nucleophilic and/or reductive cellular status. Thus, we propose a new concept : RSS protect cells from electrophilic insults, and excessive exposure to electrophilic species appears to deplete these RSS, which leads to greater susceptibility to electrophile-dependent toxicity.

2-PTS-5 Formation mechanisms and redox-regulatory functions of reactive persulfide and protein polysulfur species

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Cysteine persulfide is known to be a physiological metabolite formed ubiquitously in various organisms. We recently identified appreciable generation of cysteine persulfide in cultured cells and in vivo. These reactive sulfur species like persulfides and related species (e.g., hydropolysulfides) were potent scavengers for reactive oxygen species and showed a strong redox signaling regulatory function via electrophile thiolation. The best example is a chemical and biological interaction of persulfide species with the endogenously generated electrophile 8-nitro-cGMP. The biological relevance of cysteine persulfides/polysulfides is now increasingly recognized as essential structural residues or prosthetic components of many proteins and enzymes, which may include metal ligands most typically observed with iron sulfur clusters. Surprisingly, a clear translation-coupled cysteine polysulfuration is revealed herein and its incorporation into proteins ubiquitously occurs among different organisms. Clarification of molecular mechanisms of biosynthesis and physiological functions of polysulfur proteins may potentially promote a paradigm shift of molecular and chemical biology, opening up to a new era of innovation in the redox biology.

2-PTS-6 Mutual redox-modification of NO and reactive sulfur species

Yasuo Watanabe

Dept. Pharmacol., Showa Pharmaceutical Univ.

Hydropersulfides on protein Cys residues (S-sulfhydration) play important roles for modulation of protein structure and functions. The reactive sulfur species like cysteine hydropersulfides is generated by cystathionine γ-lyase (CSE), which in turn may contribute to other cysteine hydropersulfides derivatives of proteins in cells. Indeed, a large number of S-sulfhydrated proteins have been identified using proteomic methods in CSE-operated cells. Recent results have identified Ca^{2+}/calmodulin-dependent protein kinases (CaMKs) and nitric-oxide synthases (NOSs) as S-sulfhydrated enzymes. Interestingly, treatment of CSE or CaMKs with S-nitrosocysteine results in an inactivation of each enzyme activity via its S-nitrosylation. Thus, reactive sulfur and NO signaling are mutually regulated through the S-sulfhydrated- and S-nitrosylated-modification of specific proteins. To clarify important biological function of hydropersulfides in cells, the fluorescent probes are useful tools for their visualization. Recent research has assigned redox dependent functions to specific hydropersulfides including biosynthesis, control of enzyme activity, and role as redox sensor.

1-S-01-1 The regulatory functions of microglia in brain development and their application possibility in the drug development and therapeutics

Kaoru Sato

Lab. Neuropharmacol., Div. Pharmacol., NIHS

CNS microglia have long been considered as resident immune cells, which are activated in response to pathological events. In pathological conditions, they change their morphology to an amoeboid shape, acquiring activation-specific phenotypes, such as chemotaxis, phagocytosis, and secretion of inflammatory cytokines in the surrounding environment, thereby determining the extent of inflammation. However, There is increasing evidence that microglia also play diverse roles in brain development. For example, we recently clarified that activated microglia accumulate in the subventricular zone and enhanced neurogenesis and oligodendrogenesis during the early postnatal period. After P14, they dispersed to white matter where they became more ramified. More recently, we clarified that microglia significantly enhanced the functional maturation of blood brain barrier. In both situations, microglia reveal their regulatory effects through regulating the cytokine/chemokine dynamics of surrounding environments. We may therefore achieve more efficient therapeutic effects if we clarified the combinations of cytokine/chemokines essential for the regulatory functions of microglia in brain development.

1-S-01-2 Regulation of Tissue Stem Cells in Hair Follicle Regeneration and Aging

Emi Nishimura, Nan Liu

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The hair follicle is a mini-organ of the skin that is specialized to grow hair. Somatic stem cells including hair follicle stem cells and melanocyte stem cells and their progeny interact and regulate each other to grow a pigmented hair. To understand the mechanisms of senescent baldness and hair graying, we have studies the aging-associated changes in murine and human hair follicles and found that the fate of somatic stem cells becomes dys-regulated by aging thereby causing the aging phenotypes. Furthermore, we found that the control of the key step can prevent the expression of the hair follicle aging. In this symposium, mechanisms of tissue and organ aging and potential strategies for anti-aging will be discussed.

1-S-01-3 Modeling human neurological diseases using iPSC-derived neural stem cells with subtype specificity

Wado Akamatsu

Center for Genomic and Regenerative Medicine

Although in vitro disease models employing human pluripotent stem cells (PSCs) have great potential to clarify the association of neuronal subtypes with disease, it is currently difficult to compare various PSC-derived subtypes due to variety of cultivation procedures. Here, we report a culture system to control the regional identity of PSC-derived neurons along the anteroposterior (A-P) and dorsoventral (D-V) axis just adding several small molecules and growth factors. In the ALS iPSC-derived neurons, neurite swellings were observed preferentially in neurons with the characteristics of the ventral spinal cord. In contrast, the numbers of p-tau+spots are increased in neurons with forebrain characteristics derived from Alzheimer's disease (AD) patients. These data suggested that specific brain regions are preferentially damaged in most neurological diseases and regional specificity of neural stem cells must be an important factor for neural disease modeling with iPSCs.

1-S-01-4 Direct Cardiac Reprogramming for Heart Regeneration

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Despite recent progress in cardiovascular biology and medicine, heart failure remains a leading cause of death and new therapies are highly demanded. Due to the limited regenerative capacity of cardiomyocytes, regenerative therapy has emerged as an attractive approach for the treatment of heart failure, and direct cardiac reprogramming from fibroblasts might be a powerful strategy toward this goal. We first reported that a combination of three cardiac-specific transcription factors, Gata4, Mef2c, and Tbx5 (GMT), could directly reprogram mouse fibroblasts into cardiomyocyte-like cells in vitro (Ieda et al. Cell, 2010). Subsequently, we and others demonstrated that gene transfer of the cardiac reprogramming factors in the mouse infarct hearts could directly convert endogenous cardiac fibroblasts into cardiomyocyte-like cells in vivo (Inagawa et al. Circ Res, 2012). In human, addition of Myocd and Mesp1 to GMT (GMTMM) reprogrammed human cardiac fibroblasts into cardiomyocyte-like cells (Wada et al. PNAS, 2013, Muraoka et al. EMBO J, 2014). Although more refinements are needed, these findings might inform new regenerative strategies for the treatment of heart failure (Sadahiro et al, Circ Res, 2015).

1-S-02-1 Development of endothelial dysfunction-induced aortic dissection model and search for a preventive strategy

Yuki Izawa-Ishizawa[1], Masaki Imanishi[2], Yoshitaka Kihira[1], Licht Miyamoto[3], Yoshito Zamami[2,4], Yasumasa Ikeda[1], Koichiro Tsuchiya[3], Toshiaki Tamaki[1], Keisuke Ishizawa[2,4]

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Aortic dissection (AD) is considered to be based on hypertension and degradation of media. Endothelial dysfunction also might be necessary for AD onset. However, the detailed mechanism of AD is still unknown. We have tried to develop a novel mice model showing a high incidence of AD based on pharmacologically-induced endothelial dysfunction. In our recent study, it was revealed that pitavastatin, a HMG-CoA reductase, showed endothelial protective effect and anti-inflammatory effect. Therefore we have also assessed the effect of pitavastatin on our novel AD model.C57Bl/6J mice were treated with Nω-nitro-L-arginine methyl ester (L-NAME), a nitric oxide (NO) synthase inhibitor, to induce endothelial dysfunction, and followed by the administration of angiotensin II (Ang II) and β-aminopropionitrile (BAPN). Incidence of AD and lethal rupture was significantly increased in L-NAME, Ang II, and BAPN (LAB) group compared with Ang II and BAPN (AB) alone. Administration of pitavastatin significantly increased NO production in aorta and suppressed AD onset compared to LAB group.

1-S-02-2 Underlying mechanisms for arteriosclerosis in Intermittent hypoxia or biomechanical stretch

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Atherosclerosis is caused by several factors, such as diabetes, hypertension and dyslipidemia. Among these factors, obstructive sleep apnea characterized by intermittent hypoxia (IH) during sleep, is well known as a risk factor, but its mechanisms have not been elucidated. Recently, we have shown that IH directly induces rat aortic smooth muscle cells (RASMCs) proliferation which is important step in progression of atherosclerosis with up-regulation of several epidermal growth factors and erbB2 receptor mRNAs. In addition, erbB2 receptor inhibitor suppressed IH-induced RASMC proliferation.

It is thought that acute aortic dissection is accompanied by vascular smooth muscle cell death, because aortic dissection is characterized by aortic medial degeneration. We investigated the effects of mechanical stretch, which mimics an acute rise in blood pressure, on RASMC death. Mechanical stretch induced RASMC death and activation of c-Jun N-terminal kinase and p38. In addition, mechanical stretch-induced RASMC death was suppressed by azelnidipine.

Further investigation of the effects of IH and mechanical stretch on vascular smooth muscle cells may be clews for establishing treatments or prophylaxes of atherosclerosis and acute aortic dissection.

1-S-02-3 A new strategy for the treatment of metabolic disease, with a focus on MGAT inhibitor

Chihiro Okuma[1,2], Takeshi Ohta[1], Shinichi Kume[2], Hironobu Tadaki[1], Hiromi Hamada[1], Tomohiro Oda[1], Hideyuki Taniuchi[1], Yukihito Ishii[1], Tatsuya Ishigure[1], Shohei Sakata[1], Jun Nishiu[1], Makoto Kakutani[1]

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Atherosclerosis is accelerated by metabolic diseases. From this point of view, to efficiently and effectively manage the risk factors is regarded as important for prevention of atherosclerosis.Monoacylglycerol acyltransferases (MGATs) catalyze in the first step of triglyceride synthesis and are involved in dietary fat absorption. Recently, it has been shown that MGATs are closely associated with metabolic diseases such as obesity, type2 diabetes and nonalcoholic fatty liver disease, which are risk factors of atherosclerosis.JTP-103237 is a novel MGAT2 inhibitor generated in our institute. In studies with diet induced obesity mice, JTP-103237 decreased body weight gain in a dietary fat dependent manner and improved glucose tolerance. In addition, it decreased hepatic TG contents and partly increased energy expenditure. In the studies with high sucrose diet fed mice, JTP-103237 decreased TG contents, de novo lipogenesis and SREBP1c expression in liver. Moreover, it decreased plasma cholesterol levels.Although there is room for further investigation including prevention study in atherosclerosis models, JTP-103237 is expected to be a unique drug for the treatment of metabolic diseases and new option for management of atherosclerosis.

1-S-02-4 Progression of arteriosclerosis from a point of view of ROCK: a translational study

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Recently, there is accumulating evidence that the increase on Rho-associated coiled-coil forming kinase (ROCK) activity could evoke the onset and progression of cardiovascular disease. Therefore, we sought to determine the clinical significance of vascular and leukocyte ROCK activity in humans and roles of ROCK1 and/or ROCK2 in cardiovascular diseases using ROCK1 and ROCK2 knockout mice. We have found in clinical studies that ROCK activity is increased in smokers, a human model of oxidative stress, ROCK activity substantially correlates with endothelial dysfunction, and aging-related increase on aortic stiffness is mediated via vascular ROCK activity. We further found several mechanisms of ROCK-mediated atherosclerosis by in vitro and in vivo studies. Indeed, recent studies have shown the functional difference between the two isoforms, hence, we have focused on the downstream signaling pathway on ROCK1 and/or ROCK2 using not only ROCK1 and ROCK2 knockout mice but also cells isolated from these mice. In addition, we are working on ROCK-related translational study including cellular, animal, and clinical studies, viz. bench to bedside study. In this symposium, we would show the data of our translational study.

1-S-03-1 New functions of reactive astrocytes

Schuichi Koizumi

Dept. Neuropharmacol., Interdiscip. Grad. Sch. Med., Univ. Yamanashi

Astrocytes become reactive in the several inflammatory and neurodegenerative diseases, which control a big variety of brain functions including excitability and morphology of neurons. “Reactive astrocyte” is a well used term in several pathological conditions, many of which are thought to be correlated to pathogenesis of the diseases. However, reactive astrocytes have a big variety of forms, some of which are thought to be rather beneficial for the brain functions. Here, we show that two types of reactive astrocytes after brain ischemia using middle cerebral artery occlusion (MCAO) model of mice. After a brief non-lethal MCAO (preconditioning), astrocytes become reactive and express P2X7 receptors that induced ischemic tolerance. This astrocytic P2X7 receptor was a required for the induction of ischemic tolerance. In addition, we previously showed that an increase in Ca2+ excitability in astrocytes in the primary somatosensory cortex is a primary cause of synaptic remodeling in a model of neuropathic pain in mice. Unlike preconditioning-evoked ones, these astrocytes are different form PC-induced neuroprotective reactive astrocytes, but rather synapse-forming phenotype. We also discuss molecular based mechanisms underlying distinct phenotype of reactive astrocytes.

1-S-03-2 Sexual dimorphism in immune-neuronal signaling in pain hypersensitivity

Michael Salter

Northbridge Chair in Paediatric Res., Hosp. Sick Children; Dept. Physiol, Univ. Toronto

Neuron-microglial interactions are recognized as key for physiological and pathological processes in the CNS. Microglia have been found to play a causal role in neuropathic pain resulting from peripheral nerve injury, and a core neuron-microglia-neuron signaling pathway has been elucidated. Within the spinal cord, microglia suppress neuronal inhibition by a cascade involving activation of microglial P2X4 receptors causing the release of brain derived neurotrophic factor (BDNF). BDNF acts on trkB receptors which leads to a rise in intracellular [Cl^-] in dorsal horn nociceptive output neurons, transforming the response properties of these neurons. This core signaling pathway has been extensively characterized, in studies using male mice. We have recently discovered that microglia-neuron signaling is dispensable in female mice. Rather, pain hypersensitivity in female mice depends upon the adaptive immune system, likely upon T cells. Despite this profound difference in cellular mechanisms, pain hypersensitivity in female mice is as robust as that in male mice. Taking into consideration sex differences in the spinal immune-neuronal signaling has important implications ranging from diagnostics, to therapeutics, to prevention of chronic pain.

1-S-03-3 A crucial role of spinal astrocytes in chronic itch

Makoto Tsuda

Dept Life Innov., Grad. Sch. Pharmaceut. Sci., Kyushu Univ.

Chronic itch is an intractable symptom of inflammatory skin diseases, such as atopic dermatitis. Recent studies have revealed the selective neuronal pathways for itch sensations; however, the mechanisms by which itching turns into a pathological chronic state are poorly understood. In my talk, I will show our findings indicating that astrocytes are markedly activated in the dorsal horn (SDH) of spinal segments corresponding to the regions of itchy skin. STAT3 was selectively activated in SDH astrocytes and that conditional disruption of astrocytic STAT3 activation prevented the astrocytic activation and chronic itching. Pharmacological inhibition of STAT3 in the SDH ameliorated the fully developed chronic itch. Furthermore, atopic dermatitis mice exhibited an increase in scratching elicited by intrathecal administration of gastrin-releasing peptide, and this enhancement was normalized by suppressing STAT3-mediated reactive astrocytes. Moreover, we identified lipocalin-2 (LCN2) as an astrocytic STAT3-dependent upregulated factor that was crucial for chronic itch. Therefore, reactive astrocytes in the SDH with activated STAT3 play a pivotal role in chronic itch by enhancing spinal itch signaling by LCN2 and may represent a previously unrecognized target for treating chronic itch.

1-S-04-1 Chemical concentration profile imaging using scanning electrochemical microscopy and scanning ion conductance microscopy hybrid system

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Scanning electrochemical microscopy (SECM), a type of scanning probe microscopy, has been used to characterize and image the local chemical concentration and flux by scanning sample surfaces with an ultra-micro electrode (UME). It is effective to evaluate a single cell respiration activity and neurotransmitter release, but the resolution is related with the electrode size and electrode-sample distance. To improve the resolution of SECM, we adopted distance control system of scanning ion conductance microscopy (SICM) and fabricated SECM-SICM probe. SICM is a promising technique for non-contact topography imaging under physiological conditions. SICM uses a nanopipette as a scanning probe to detect ionic current between an electrode inside the pipette and that located in a bath. We have developed an extremely quick (2 min) and simple process with a high success rate for making SECM-SICM probe. The method allows fabrication of SECM-SICM probe with a radius ranging from 10 nm to 1 um. In this presentation, we will discuss about cell membrane permeability measurement and local neurotransmitter detection using SECM-SICM.

1-S-04-2 In vivo real-time measurement of local drug concentrations by using diamond microelectrode

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For pharmacological studies, it is important to analyze local concentrations of systemically administered drugs in in vivo various organs and tissues. Conventional methods do not accomplish this because they require considerable analyte quantities and have low sampling rates. They also cannot address how change of drug concentrations correlates with target cell or tissue functions over time. Therefore, we designed a system equipped with two different sensors. One is a boron-doped diamond microsensor, which semi-quantitatively detected change of concentrations of vascularly applied ototoxic diuretic, bumetanide, in the extracellular fluid of the cochlea of live guinea pigs. The time resolution ranged within several seconds. The other, a glass microelectrode simultaneously monitored the endocochlear potential underlying hearing. This multi-sensing system can be applied to monitor other drugs and electrophysiological functions in numerous tissues and organs and promote the pharmacological researches.

1-S-04-3 Microfluidic devices for cell culture and cell-based assay in drug discovery

Shinji Sugiura, Taku Satoh, Kazumi Shin, Toshiyuki Kanamori

Biotech. Res. Inst., AIST

Microfluidic devices are expected to create a new opportunity in drug discovery owing to their advantageous characteristics, including precise fabrication of small structures, easy replication of the fabricated structure, precise manipulation of small volume liquid, and usage of small amounts of expensive reagents. Generally, cell culture in a microfluidic device is carried out by medium perfusion using syringe pumps. However, liquid handling using syringe pump is cumbersome. To address this issue, we developed a pressure driven perfusion culture system, in which multiple liquids could be handled by simply applying pressure in the liquid reservoir. This is a convenient system to create different culture conditions in a single microfluidic device. We have applied pressure driven perfusion culture system to drug dose response assay, circulation culture system, and multi-organs culture system.

1-S-04-4 Integration of in vitro data using pharmacokinetic model

Hiroyuki Kusuhara

Grad. Sch. of Pharm. Sci., the Univ. of Tokyo

Because of its difficulty, bridging from preclinical to clinical studies is referred to as Devil River. Species difference in the pharmacokinetics between animals and humans is one of the obstacles. Measurement of human kinetic parameters will bridge the gap in the pharmacokinetics of drugs. Physiologically-based pharmacokinetic (PBPK) model, where the anatomical and physiological structure of the body is considered, is frequently used for pharmacokinetic prediction of drugs. We demonstrated that combining in vitro data measured using human derived materials, and scaling factors could successfully describe the plasma concentration time profiles of pravastatin following intravenous and oral administration in humans using PBPK model. Furthermore, we newly introduced a newly developed parameter estimation method, cluster newton method (CNM) which automatically prepares multiple initial parameter sets when the researchers just set the rages of the parameters, and provides multiple sets of fitted parameters as solution. We applied this method to the quantitative analysis of clinically-reported drug-drug interactions using PBPK model. PBPK model is a good plat home for bottom-up and top-down approaches in the pharmacokinetic analysis of drugs.

1-S-05-1 Health foods and weight management

Yi-Wen Chien

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Many different dietary approaches have been studied in an attempt to achieve healthy, sustainable weight loss among individuals with overweight and obesity. Meal replacement, low carbohydrate-low glycemic index (GI) diet, high protein intake, and moderate fat consumption have shown some positive effects on weight maintenance. However, the results are controversial. Some findings from our studies demonstrate that weight loss related to dairy product intake is due to the combination of an energy-restricted diet with consumption of dairy products. Using milk or yogurt contained conjugated linoleic acid (CLA) on weight loss program had more significantly reduced in body weight, body mass index (BMI), and body fat percentage. A new supplement with hydroxycitric acid (HCA) and chromium (Cr) significantly reduced weight gain and body fat accumulation in diet-induced obesity rats. Further, the commercial product containing garcinia cambogia extract, yerba mate extract and guarana extract, can be helpful in weight control along with nutrition education and maintaining high-density lipoprotein cholesterol level. Our results can provide more information about a proper choice of health foods for weight loss or maintain programs.

1-S-05-2 Lifestyle-related diseases improved by functional food

Susumu Yoshino, Riyo Awa, Yasuo Miyake, Nobuaki Ohto, Hiroshige Kuwahara

Maruzen Pharmaceuticals Co., Ltd.

As Lifestyle-related diseases, particularly obesity and the allied diseases continue increasing significantly around the world, the development of effective prophylaxis and its enlightenment are important issues. Since the utilization of food component, addition to daily physical exercise, is considered to be an effective countermeasure against obesity, the development of functional food collects big attention recently. Kaempferia parviflora (KP), known as black-ginger or krachai-dum, is a plant that belongs to the Zingiberaceae family and has been used as a folk medicine for long time. KP includes polymethoxyflavone, which has a variety of beneficial activity.We previously demonstrated that KP suppresses the weight gain and fat accumulation and increase the energy consumption in mice fed high-fat diet. We also demonstrated that KP increased the energy consumption in human. These results suggest that KP is a useful functional food in order to prevent obesity.In this symposium, I will outline the anti-obesity effect and the mechanism of KP as an example of functional food, and further discuss the benefits and future challenges of functional food.

1-S-05-3 Improvement of bone metabolism by functional foods

Nobuo Izumo, Yasuo Watanabe

General Health Medical Center, Yokohama University of Pharmacy.

In the life related diseases, it has been well documented that the hypertension, hyperlipidemia and hyperglycemia cause the sudden death and the chronic severe diseases. However, the dysfunctions of bone metabolism induced by life style have not been fully understood. And also it has been reported that such dysfunctions can be caused by not only aging but also lack of exercises. Moreover, interestingly, the young sportswomen have several types of less developing of bone growth due to the deviation of nutrition. In our recent studies, we examined the effects of several functional foods and water on bone metabolism. Then we evaluated that some substances (e.g. hydrogen water and lactoferrin) significantly stimulated the ossification and attenuated osteoclastic. These substances also inhibited the steroid induced bone dysfunctions. Furthermore, these substances did not show any severe adverse evidences. These results indicate that the daily taken healthy substances can prevent the bone metabolic dysfunction induced by irregular life style. We also discuss about the protection of bone metabolic dysfunction based on the pharma-food interaction in this symposium.

1-S-05-4 Dietary supplements for hepatoprotection

Jane Chao

School of Nut. and Health Sciences, Taipei Med. Univ., Taipei, Taiwan

The mortality rate for liver and intrahepatic bile duct cancer was 34.9 per 100,000 in2014. Therefore, hepatoprotection is a major approach to prevent the development liverdisease and hepatocellular carcinoma in Taiwan. Some dietary supplements, such as fish oil,nicotinic acid, flaxseed, and active components in herbs, have been considered to be potentialfor hepatoprotection via antioxidant, anti-inflammatory, and antifibrotic activities. The n-3polyunsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic acids rich in fishoil, showed hepatoprotective activity against the development of non-alcoholic fatty liverdisease by modulating lipogenesis and lipolysis. Supplementation with nicotinic acid andflaxseed showed to protect liver damage against oxidative stress via the improvement ofantioxidant defense. Ginseng extract and ginsenosides are shown to attenuate liver fibrosis byregulating fibrogenesis. Hot water extracted Lycium barbarum and Rehmannia glutinosa protectedagainst necrotic damage in the liver and suppressed liver fibrosis. Therefore, appropriate dietarysupplements with hepatoprotective activity could improve liver damage and inhibit the developmentof liver disease.

1-S-06-1 Kidney and amino acids in blood pressure regulation, with special attention on dopamine, GABA and salt intake

Midori Yatabe, Junichi Yatabe, Atsuhiro Ichihara

Dept. Endocrinology and Hypertension, Tokyo Women's Med. Univ.

Amino acids and metabolites can serve as signaling molecules. Dopamine is a metabolite of amino acid tyrosine via L-dopa. In the kidney, dopamine is synthesized in the proximal tubule and increases sodium excretion during dietary NaCl load. Renal antihypertensive effects of dopamine are exerted mostly via D1 receptor. GRK4, a receptor kinase, modifies renal dopaminergic signaling, and genetic polymorphisms of GRK4 are implicated in the pathogenesis of salt sensitivity in humans. Another amino acid-related signaling molecule is gamma aminobutyric acid (GABA). GABA is a non-proteinogenic amino acid, has antihypertensive properties, and is synthesized in the kidney. A part of anti-hypertensive effect of GABA may be due to its renal actions because GABA receptors are identified on renal tubules, and GABA induces natriureis and diuresis. Salt increases urinary excretion of various amino acids, and plasma concentrations of some amino acids are shown to be different in hypertensive models. Advances in technology has made amino acid research more active in recent years, and new findings can be expected to unravel novel mechanisms of renal blood pressure regulation.

1-S-06-2 Multiphoton Imaging of Renal Function

Daisuke Nakano

Department of Pharmacology, Kagawa University, Kagawa

Urine output is widely used as a criterion for acute kidney injury (AKI) diagnosis. Although a number of investigations have identified potential mechanisms of AKI, regulation of urine flow has not been evaluated. We have developed the methods to evaluate changes of urine flow as well as multiple renal function by intravital imaging to enable real-time monitoring by multiphoton microscopy. The tubular flow rate was measured by freely filtered dye (FITC-inulin or Lucifer yellow) that was time-dependently declined in the experimental acute kidney injury models; it was already slower at 2 h in LPS-injected mice compared with saline-injected mice, while the blood pressure and GFR were normal. Mitochondrial membrane potential in the tubules depends on the peritubular capillary flow rather than tubular flow. These analysis could be the useful tool to evaluate the renal function in the live animals, and shed light on the black box in the renal physiology.

1-S-06-3 Kidney as a sensor and a regulator of internal mileu

Tatsuo Shimosawa

Dept. Clinical Lab. Univ. Tokyo Hospital

Physiological function of kidney is not only excrete waste but sense the changes of chemical signals and mechanical signals and regulate CNS and cardiovascular functions. In this symposium, I focus on sympathetic regulation of kidney function and kidney-derived regulation of sympathetic tone. Catecholamine regulate renin-angiotensin axis in the kidney to regulate glomerular pressure as well as ion exchanges in the tubules. Recently I clarified that catecholamine regulate sodium reabsorption via beta-2 receptor and sodium-chloride co-transporter independent from angiotensin II. Moreover, we observed cardiac diastolic dysfunction in high salt loaded-rat. In this rat model, denervation of both afferent and efferent sympathetic nerve in the kidney reversed cardiac dysfunction independent from blood pressure or cardiac remodeling. These data suggest that renal efferent sympathetic nerve regulate sodium homeostasis in contrast afferent nerve sense sodium level to convey signal to the heart possibly via CNS. It is well known that kidney senses blood pressure, ion mileu, oxygen pressure and other signals and regulate homeostasis. Taken together, kidney could be a center for giant data of whole body and control tower of the homeostasis.

1-S-06-4 WNK signaling and KLHL3/Cullin3 ubiquitin ligase complex in salt-sensitive hypertension

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Pseudohypoaldosteronism type II (PHAII) is a hereditary disease characterized by salt-sensitive hypertension. Mutations in with-no-lysine kinase 1 (WNK1) and WNK4 genes are found to cause PHAII. It was demonstrated that WNK kinases constitute a signaling cascade with OSR1, SPAK, and the SLC 12a transporter family, including thiazide-sensitive NaCl cotransporter (NCC). The WNK-OSR1/SPAK-SLC12a signaling cascade exist in the kidney and vascular smooth muscle cells, and physiologically regulates salt-sensitivity, i.e., urinary sodium excretion and arterial tone by a various hormonal and dietary factors. In 2012, two additional genes responsible for PHAII, Kelch-like 3 (KLHL3) and Cullin 3 (CUL3), were identified. WNK1 and WNK4 was shown to be substrates of KLHL3-CUL3 E3 ubiquitin ligase complex. The loss of interaction between KLHL3 and WNK4 induces increased levels of WNK kinases due to impaired ubiquitination, resulting in PHAII. These results implicated that WNK signaling is physiologically regulated by KLHL3/CUL3-mediated ubiquitination. In this session, we would like to present the pathophysiological roles of the WNK signaling cascade in the kidney and vascular smooth muscle cells and the regulation of WNK signaling by KLHL3 and CUL3.

1-S-07-1 Intracellular signaling of Sema3A through phosphorylation cascade

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Sema3A, one of vertebrate secreted semaphorins, is a repulsive axon guidance molecule. Sema3A inhibits the neurite outgrowth of dorsal root ganglion neurons and hippocampal axons. Sema3A also regulates the dendritic formation of cortical and hippocampal neurons. The receptor for Sema3A is the complex of Neuropilin-1 and Plexin-A. This complex activates three different intracellular signaling : small-G-protein cascade, phosphorylation cascade, and oxidation signaling. We will introduce our recent findings of the phosphorylation cascade and discuss therapeutic targets of this signaling.

1-S-07-2 Involvement of semaphorins in immunological disorders.

Atsushi Kumanogoh

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Semaphorin was originally identified as neural guidance factors. Recent research on semaphorins demonstrated that these proteins play pleiotropic functions in immunological regulation, angiogenesis, tumor metastasis, and bone metabolism. In particular, semaphorins involved in various phases of immune responses are referred to as "immune semaphorins". Accumulating evidence demonstrates that immune semaphorins play important roles in the pathogenesis of autoimmune diseases. We here present the pathological involvement of semaphorins and their receptors in immunological disorders, including the roles of Semaphorin 4D in rheumatoid arthritis.

1-S-07-3 The regulation of bone remodelling by semaphorin

Tomoki Nakashima

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Bone is constantly renewed by the balanced action of bone formation and bone resorption. This restructuring process called "bone remodeling" is important not only for normal bone mass and strength, but also for mineral homeostasis. Bone remodeling is stringently regulated by communication between bone component cells such as osteoclasts, osteoblasts and osteocytes. An imbalance of this process is often linked to various bone diseases. Recently, we identified that semaphorin molecules have a crucial role in the regulation of bone remodeling. The regulatory mechanisms provide a scientific basis for future therapeutic approaches to bone diseases.

1-S-07-4 Revisiting the structure–activity relationship of semaphorin 3A

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Semaphorin 3A (Sema3A) is a prototypic soluble semaphorin that activates A–type plexin on cell surface, with a help of its high–affinity co–receptor neuropilin–1 (Nrp–1). Although a crystal structure exists for a ternary complex containing minimum fragments of these three components (Sema3A, Plexin A2, and Nrp–1), we still don't know exactly how Sema3 A activates Plexin on cell surface. In addition, although it has been known since 1997 that Sema3A undergoes complex proteolytic processing that regulates its activity, the molecular mechanism underlying such regulation has not been rationalized in the context of the structure. In order to reinvestigate the structure–activity relationship of Sema3A, we established a high–throughput cell collapse assay system using non–neuronal cells. Systematic evaluation of various mutant Sema3A proteins revealed a presence of multiple "active sites" distributed over the entire molecule, enabling us to design a Sema3A variant that is highly active and more stable than the wild type protein. Combined with plexin A1 ectodomain structures deduced from electron microscopic molecular imaging, a revised model of Sema3A–induced plexin activation on cell surface is constructed.

1-S-08-1 The collecting lymphatic vasculature: regulation and roles in pathophysiology

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Studies in mice recently revealed that infection–induced tissue damage in the mesentery causes hyperpermeable, leaky lymphatic collecting vessels and consequent persistent alterations in tolerance and immunity normally programmed by mesenteric lymph nodes. To address whether analogous changes might occur in human IBD, we established a multistep, 3D imaging approach to characterize mesenteric tissue from Crohn's disease resections or non–IBD subjects. In Crohn's disease specimens, lymphocyte rich aggregates were observed to markedly constrict the contractile collecting lymphatic vessels that drain to and from lymph nodes. These were associated with a striking intimal hypertrophy of adjacent veins. In other literature, collecting vessel constriction has been experimentally shown to promote leaky collecting vessels and venous collapse. Thus, the imaging data in Crohn's disease tissue are consistent with the possibility that lymph and venous outflow from the intestinal wall may be sufficiently altered in Crohn's disease to promote leaky collecting vessels that might in turn prevent normal lymph node–mediated regulation of immunity. Data will be presented that implicates IRF4+ CCR7+ dendritic cells in control of collecting vessel permeability.

1-S-08-2 Roles of signal network during the endothelial–to–mesenchymal transition (EndMT)

Tetsuro Watabe

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Endothelial cells undergo differentiation into mesenchymal cells during various physiological and pathological processes including heart valve formation and cancer progression, respectively. However, the molecular mechanisms that regulate such endothelial–to–mesenchymal transition (EndMT) remain to be elucidated. Here we show that TGF–β plays important roles during mesenchymal differentiation of endothelial cells. By addition of TGF–β2, MS–1 endothelial cells underwent mesenchymal transition characterized by increased expression of various mesenchymal markers such as SMA. We found that TGF–β2–induced EndMT of MS–1 cells is dependent on the activation of Rho signals. We found that TGF–β2 induces the expression of myocardin–related transcription factor–A (MRTF–A) and its nuclear accumulation in MS–1 cells and that MRTF–A is required and sufficient for TGF–β 2–induced SMA expression. These results indicate that activation of Smad signals by TGF–β2 have dual effects on the activation of Rho signals and MRTF–A leading to the mesenchymal transition of MS–1 endothelial cells. Taken together, these findings suggest that TGF–β2 activates multiple transcriptional and signaling networks during mesenchymal transition of endothelial cells.

1-S-08-3 Platelet activation blocks misconnection of lymphatic to blood vessels in mouse peripheral tissues via TGF–β signals

Masanori Hirashima

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Lymphatic endothelial cells (LECs) are derived from venous endothelial cells during embryogenesis and subsequently form a vasculature separate from blood vessels in peripheral tissues. Recent studies indicated a critical role of platelets activated by LECs in proper separation between these two vascular systems. However, how platelets keep lymphatic vessels separate from blood vessels remains unknown. Here, we show that abnormal lymph–blood connections randomly occur in embryonic back skin of $Plcg2^{-/-}$ mice lacking platelet activation, suggesting a critical role of platelets during lymphatic vessel elongation. *In vitro* analysis showed that platelets or activated platelet–conditioned medium inhibits LEC migration and proliferation in a Syk– or Plcg–dependent manner and also induces an immediate filopodial retraction of LECs. These effects were inhibited by an inhibitor of TGF–β signals, while TGF–β induced a filopodial retraction of LECs. *In vivo* analysis indicates the role of TGF–β in keeping lymphatic vessels separate from blood vessels. These results indicate a novel role of platelets in partitioning blood and lymphatic vascular compartments by promoting LEC retraction in mouse peripheral tissues.

1-S-08-4 Roles of Prostaglandins in regulation of plasticity of lymphatics and lymph nodes

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Angiogenesis is upregulated by prostaglandins (PGs), however, little is known about their involvement in lymphangiogenesis. In chronic inflammation, lymphangiogenesis detected by double immunostaning of VEGFR-3 and LYVE-1 was upregulated via COX-2 and EP3/4 signaling during the development of granulation tissues. The lymphatic system is an important route for cancer dissemination, and lymph node metastasis (LNM) serves as a critical prognostic determinant in cancer patients. A murine model of Lewis lung carcinoma (LLC) cell metastasis revealed that COX-2 is expressed in dendritic cells (DCs) from the early stage in the lymph node subcapsular regions, and COX-2 inhibition markedly suppressed mediastinal LNM. Stromal cell-derived factor-1 (SDF-1) was elevated in DCs before LLC cell infiltration to the lymph nodes, and a COX-2 inhibitor, an SDF-1 antagonist, and a CXCR4 neutralizing antibody all reduced LNM. LNM was reduced in mice lacking EP3, and stimulation of cultured DCs with an EP3 agonist increased SDF-1 production. Accumulation of regulatory T cells and lymph node lymphangiogenesis was also COX-2/EP3-dependent. These results indicate that COX-2 and EP signaling modulate plasticity of lymphatics and lymph nodes in pathological settings.

1-S-09-1 A murine model of Japanese cedar pollinosis for studying mechanisms and potential biomarkers of allergen-specific immunotherapy

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<Objective>Sublingual immunotherapy (SLIT) is considered as a potentially curative treatment for allergic diseases such as Japanese cedar pollinosis (JCP), in which patients were exposed with JC pollen every spring. However, the mechanisms and biomarkers involved remain to be elucidated. In this study, we established a murine model of JCP and evaluated the efficacy of SLIT. <Methods>BALB/c mice were intranasally sensitized and challenged with Japanese cedar pollen crude extract (JC) and were challenged every 5 weeks to induce allergic nasal responses, which mimics the JCP. The mice were treated with JC-SLIT between the challenges and then the therapeutic effect was assessed by number of sneezes, antigen-specific T cell responses, antibodies, and histology of nasal mucosa.<Results>JC-SLIT significantly reduced the JC-specific allergic reactions, with long-lasting effect even after completion of treatment (carry-over effect). <Conclusion>We have generated a mouse model of JCP that is useful for studying mechanism and biomarkers of SLIT.

1-S-09-2 Establishment of a model mouse of food allergic enteropathy

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Research Center for Food Safety and Dept. Applied Biol. Chem., The Univ. Tokyo

Food allergy induces uncomfortable symptoms and in some cases may be a life-threatening disease. Our interests have concentrated on clarifying its detailed mechanisms of establishment of inflammation and of its recovery. To study the mechanism, we established a food allergic mouse model using OVA23-3 mice, which express OVA-specific T cell receptor transgenes. OVA23-3 mice show severe enteropathy, weight loss, and increase of serum OVA-specific IgE by only feeding with EW-based diet without adjuvant. By further continuous feeding with the diet, they recover from inflammation, although in mesenteric lymph nodes, inflammatory responses are maintained. These results suggest that our model has unique characteristics to enable us to investigate the mechanism from start of food allergic enteropathy to attenuation of the inflammation developed in whole body upon feeding with OVA. Thus, we have analyzed roles of both intestinal and systemic tissues of EW-fed OVA23-3 mice throughout the experimental period monitoring IL-4 producing OVA-specific CD4+ T cells responses as a causative factor. We found each tissue played different roles in inflammatory or regulatory responses. We are now examining relation between intestinal and cutaneous responses to OVA using this model.

1-S-09-3 Analyses of asthma pathogenesis by focusing on IL-33

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IL-33, one of damage-associated molecular patterns (DAMPs), is extracellularly released when cells such as airway epithelial cells are damaged. Because IL-33 is detected in asthma lung in large amounts, and IL-33 activates various leukocytes including not only Th2 cells but also type 2 innate lymphoid cells (ILC2), IL-33 could be involved in asthma pathogenesis by orchestrating the innate and acquired immunity. It is expected that strategies to suppress IL-33 production may lead to regulation of asthma. However, production mechanisms of IL-33 have not been unclear. Using murine models of ovalbumin-induced airway inflammation, we have reported that IL-33 was involved in airway inflammation and remodeling, and Th2 cytokine production. We found that the IL-33 production from the airway epithelial cells was glucocorticoid-sensitive, and induced by not only the innate but also acquired immune mechanisms. Because anti-IgE neutralizing antibody suppressed the IL-33 production, the specific antigen may be recognized by IgE molecule followed by IL-33 production from the epithelial cells. However, mast cell/basophil-depleting antibody did not affect the IL-33 production. Further analyses of cellular and molecular mechanisms of IL-33 production may lead to new pharmacotherapy of asthma.

1-S-09-4 Rapid development of house dust mite–induced allergic inflammation

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To investigate the pathogenesis of and to develop new treatments for allergic diseases, tons of animal models have been established so far. In general, topical application of allergens to target tissues of animals previously immunized with systemic administration of corresponding allergens induces tissue-specific inflammatory responses. However it has been difficult to reproduce the developmental process of human allergy in which clinical symptoms are induced only by repeated allergen provocation to the target tissues without the systemic immunization. Although several models of airway inflammation elicited by repeated provocation of house dust mite to naive mice were reported recently, a long-term allergen challenge process was required to establish them. We here reported a new murine model in which obvious allergic airway inflammation could be induced simply by a couple of allergen application.

1-S-10-1 Neuronal axon regeneration and sulfated glycans

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Axons in the central nervous system cannot regenerate after injury. It is due to low intrinsic capacity of regeneration and emerging inhibitory molecules upon injury. Therapeutics for neuronal injuries are not currently available and have long been awaited. Chondroitin sulfate (CS) is one of the strongest inhibitors, while heparan sulfate (HS) promotes axon growth. Both CS and HS are glycosaminoglycans, which are long sulfated glycans with repeating disaccharide units, and thus have similar structures. Therefore, it is a big question why CS and HS show opposite effects on axon growth. We addressed this question to understand molecular mechanisms underlying the axon regeneration inhibition. Through identification of critical structures of CS and HS for axon growth, we found a "chance and necessity" rule of functional domains of CS and HS in their receptor organization. Natural CS has a short functional domain which monomerizes its receptor RPTPσ (receptor-type protein tyrosine phosphatase σ), blocks the autophagy flux, leads to dystrophic endball formation, and consequently suppress axon regeneration. Natural HS harbors a long functional domain that oligomerizes RPTPσ and reverses this pathway.

1-S-10-2 Hyaluronan dependent regulation of glutamate transporters

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Glutamate released to synapses must be removed after synaptic activation to terminate the signal and to protect neurons from excitotoxicity. Astrocytes are mostly responsible for the glutamate clearance. They extend thousands of thin cellular processes to approach synapses for this purpose.We found that the trimeric transmembrane transporter domain of glutamate transporters has a property to localize to filopodia tops, while their N- and C-terminal cytoplasmic tails are not required. This is a common property of trimeric transporter family members. Neither transporter activity nor astrocyte specific protein was required for this filopodia tip localization. Furthermore, the transporter core within filopodia tips strengthened the attachment of filopodia to external substrates, thereby stabilizing the filopodia. Recently we found that the process tip localization of glutamate transporter is hyaluronan dependent. For the localization, CD44, a representative hyaluronan receptor, was not required. Instead, hyaluronan synthase showed hyaluronan dependent interaction with glutamate transporters. Glutamate uptake activity was reduced by loss of process tip localization by hyaluronidase treatment, and as a result, neurons were activated glutamate dependently.

1-S-10-3 Bisecting GlcNAc modification promotes Alzheimer's disease

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Alzheimer's disease (AD) is the most common dementia and a serious issue all around the world. Recently, we found that GnT-III, a glycosyltransferase for the biosynthesis of a unique sugar modification "bisecting GlcNAc", plays a pathological role in AD. GnT-III KO mice show a dramatic decrease in production of amyloid-β (Aβ), a hallmark and the causative peptide of AD, resulting in improvement of cognitive function. We found that BACE1, an essential protease for Aβ generation, is a novel target for bisecting GlcNAc and that BACE1 is relocated from early endosomes to lysosomes without loss of its catalytic activity in GnT-III KO cells (Kizuka et al., (2015) EMBO Mol. Med.). This indicates that bisecting GlcNAc regulates Aβ production by controlling subcellular localization of BACE1.

We also revealed that bisecting GlcNAc is required for BACE 1 upregulation under oxidative stress. BACE1 protein is upregulated under various stress conditions such as aging or Aβ accumulation, and such BACE1 upregulation was abolished in GnT-III KO cells where BACE1 is degraded faster in lysosomes than wild-type cells. These data indicate that bisecting GlcNAc regulates BACE1 protein level by preventing its lysosomal degradation under stress conditions (Kizuka et al., (2015) Biochem. J.).

1-S-10-4 How we overcome blood–brain barrier? The challenge in chemotherapy against brain malignancies.

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The blood–brain barrier (BBB) generally blocks penetration of chemotherapeutics into brain stroma, which is the main obstacle in treating brain malignancies. Previously we reported that carbohydrate–mimetic peptide IF7 targets to tumor vasculature through annexin A1 and delivers anticancer drug to tumor stroma. Since IF7 crosses the endothelial cells by active transcytosis, we hypothesized that IF7 overcomes the BBB. When FITC–tagged IF7 was injected intravenously into C6 glioma tumor–bearing mice, fluorescence signals were clearly seen in the brain tumors, whereas a FITC–tagged control peptide (RQ7) with reversed IF7 sequence was not detected. When IF7 conjugated to the anti–cancer drug SN–38 (IF7–SN38) was injected intravenously into mice harboring two tumors, one subcutaneous and the other in brain, growth of both tumors was suppressed. Furthermore, IF7–SN38 suppressed brain tumor growth more effectively than that of subcutaneous tumors, regardless of cancer cell line or host mouse strain. Taken together, these results suggest that conjugates of IF7 and anticancer drug could become a novel therapeutics against brain malignancies with little side effect.

1-S-11-1 The physiological roles of hydrogen sulfide and polysulfides

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We demonstrated that cystathionine beta–synthase (CBS) is a H2S producing enzyme in the brain and that H2S facilitates the induction of hippocampal long–term potentiation (LTP) in 1996. The following year we demonstrated another role of this molecule, smooth muscle relaxation with its producing enzyme cystathionine gamma–lyase (CSE). We also found the neuroprotective effect of this molecule. This finding led to the identification of the protection of various tissues/organs against ischemia–reperfusion injury. We added a novel pathway, which produce H2S from D–cysteine, to three authentic pathways which metabolize L–cysteine. We recently found polysulfides (H2Sn) in the brain, and it activates transient receptor potential ankyrin–1 (TRPA1) channels much more potently than H2S. TRPA1 channels mediate various physiological responses including the sensory transduction, but its endogenous ligand has not been identified. H2Sn also regulates the translocation of Nrf2 transcription factor to the nucleus to upregulate aitioxidant genes. In this symposium we discuss the producing pathways of polysulfides, their physiological roles and the importance of the balance between H2S and H2Sn to regulate the activity of their target proteins.

1-S-11-2 Development of a H_2S fluorescence probe and its application to inhibitor screening for H_2S producing enzymes

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For detailed studies of the physiological functions of sulfur compounds such as H_2S and sulfane sulfur, a new method to measure their concentration in biological samples is required. For example, the existing methylene blue method and the sulfide ion–selective electrode method for measuring H_2S are destructive, requiring homogenization of samples. Therefore, we designed and synthesized a novel fluorescence probe for H_2S, HSip–1 (Hydrogen Sulfide Imaging Probe–1) (J. Am. Chem. Soc. 2011, 133, 18003). Further, HSip–1 showed a large and immediate fluorescence increment (by 50 fold) upon addition of 10 μM H_2S, whereas almost no fluorescence increment was observed upon addition of 10 mM GSH. We also applied HSip–1 to the inhibitor high–throughput screening (HTS) of a chemical library containing 160,000 compounds from The University of Tokyo, Drug Discovery Initiative, and found selective inhibitors for 3MST and CSE. Recently, we designed and synthesized a novel fluorescence probe for sulfane sulfur, SSip–1 (Sulfane Sulfur Imaging Probe–1) and this probe showed a large fluorescence increment in both cuvette and microscope measurements upon addition of 50 μM Na_2S_4 based on the intramolecular spirocyclization of the probe.

1-S-11-3 Insulin secretion and cellular stress protection in pancreatic beta–cells

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We previously reported that hydrogen sulfide inhibited glucose–induced insulin release from pancreatic islets. Underlying mechanisms for the inhibition seems to be multiple. We also demonstrated that hydrogen sulfide protected islet cells from apoptotic cell death induced by high glucose. hydrogen sulfide increased total glutathione levels and decreased the production of reactive oxygen species in the mouse beta–cell line MIN6. Finally, we found that the expression of the hydrogen sulfide–producing enzyme cystathionine gamma–lyase (CSE) was induced by stimulation with glucose in islets. This supports an idea that hydrogen sulfide may be produced in an inducible manner just like the other two gasotransmitters NO and CO. Together with our recent report that a lack of CSE induced apoptotic beta–cell death and promoted the development of HFD–induced diabetes, these findings also tempt us to suggest that hydrogen sulfide produced by CSE may be an intrinsic brake equipped within the pancreatic beta cell to inhibit insulin release and reduce cellular stress evoked by glucose, possibly via its anti–oxidant actions.

1-S-11-4 The spatial distribution of glutathione persulfide in the mouse lens

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Although glutathione (GSH) is known as the principal antioxidant in the lens, glutathione hydropersulfide (GSSH) has been recently recognized as a more effective reductant which can donate a hydrogen atom to one-electron oxidants more readily than the thiol. However, the spatial distribution and the quantification of GSSH remain to be determined. To examine spatial distributions of GSSH, we took advantage of a unique nature of an atmospheric-pressure MALDI imaging mass spectrometry (IMS) where the ionization of tissue metabolites occurs in the presence of molecular oxygen. Under such a condition, GSSH can be readily oxidized to derive glutathione S-sulfonate (GSSO3H) whose ionization efficiency is much higher than its native form, making it possible to detect this trace-amount metabolite. We found that mass intensities of GSSO3H are high in both the epithelium and the outer cortex of the lens. These results imply that hydropersulfide oxidation states might control protein functions necessary for lens transparency.

1-S-12-1 The Role of Ser/Thr Protein Phosphatases in intestinal inflammation and cancer

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In this symposium, I present a current picture of phosphatase targeting anti-cancer strategy. Protein Phosphatase 2A (PP2A) plays a central role in phosphorylation-dependent signaling pathways and is implicated as an important tumor suppresser. SET/I2PP2A is a potent inhibitor of PP2A and increased SET expression has been suggested to correlates with the poor prognosis of chronic myeloid leukemia. However, the role of SET in gastrointestinal cancer remains unknown. We found increased SET protein levels in the cancer tissues of Gan mice, which spontaneously develop gastric adenocarcinoma in an inflammation-dependent manner. We utilized lentivirus to stably express shRNA targeting SET in human gastric cancer cell lines, MKN45 and MKN74, and found that the suppression of SET expression leads to significant decrease in colony numbers in soft-agar assay and inhibits tumor growth in mouse xenograft model. SET over-expression in mouse gastric organoid model suggested SET maintains the stemness of epithelial cells. SET inhibitors effectively killed gastric cancer cells. Our results suggest that increased SET expression plays the important role in the aggravation of gastric cancer, and the possibility for SET targeting anti-cancer drugs.

1-S-12-2 The emerging role of interleukin-19 as inflammatory mediators

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It has long been appreciated that an immune response must be tightly regulated to avoid the fatal consequences of an overwhelming or inappropriate inflammation. Conversely, exposure to foreign organisms such as LPS, a cell wall component of Gram-negative bacteria, triggers the host release of inflammatory mediators that can promote the life-threatening tissue damage. Interleukin-19 (IL-19) is a member of the IL-10 family of cytokines. The last 10 years from the finding of IL-19, investigations have underlined the role of IL-19 in the immunological diseases. It is known that expression of IL-19 is increased in the epidermis of patients with psoriasis, which is a Th1 dominant disease. Increased concentration of IL-19 has also been found in the serum of patients with asthma, which is a Th2 dominant disease. Moreover, there is an increasing body of data demonstrating that IL-19 is associated with the pathogenesis of immune responses in various organs. In this symposium, we will report the recent advances in the role of IL-19 as inflammatory mediators in colitis, intimal hyperplasia and neuroinflammation.

1-S-12-3 The role of mast cell in food allergy

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Food allergy is an abnormal immune response to food proteins. The number of patients with food allergies is now increasing especially in developed countries. Symptoms affecting the skin, lung, and gastrointestinal tract include eczema, labored breathing, and diarrhea. In serious cases, hypersensitivity against food antigens may lead to systemic anaphylaxis reactions, thus threatening the life of the patient. There is no effective treatment available for these reactions. Mast cells are major players in this disease. Upon antigen consumption, mast cells release a large number of inflammatory mediators, including histamine, platelet-activator factor (PAF) and prostaglandin D_2 (PGD_2). Mast cells strongly express hematopoietic PGD synthase (H-PGDS) and produce a large amount of the arachidonic acid metabolite prostaglandin D2 (PGD2). Although PGD2 has been emerged as a mast cell-derived allergic mediator, there is uncertainty about its contribution to allergic inflammation. We have been investigating the role of prostaglandin D_2 in food allergy using genetically modified mice. In this symposium, we introduce the implication of mast cell-derived PGD_2 for food allergy and anaphylaxis, and suggest its efficacy for therapeutic application.

1-S-12-4 Roles of novel serine/threonine kinases that regulate the pathogenesis of hypertension through inflammatory reactions

Hideyuki Yamawaki

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Hypertension is often associated with inflammatory reactions through several organs including kidney, brain, heart and vasculature. However, the underling mechanisms still remain to be fully determined. Protein kinases regulate critical cellular functions including proliferation, migration, cell death and inflammation, that are important for maintaining the homeostasis of living organism. Recently, our group has discovered several protein kinases, expression of which are specifically upregulated in the tissues from experimental hypertensive animal models. In this symposium, I introduce functions of the serine/threonine kinases, namely eukaryotic elongation factor 2 kinase (also known as CaMK III) and zipper-interacting protein kinase (also known as DAPK3) especially focusing on inflammation, discuss their roles in the pathogenesis of hypertension, and propose their potential therapeutic application.

1-S-13-1 Functional role of a novel protein inhibiting mitochondrial fusion

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Several lines of evidences have revealed the possible roles of mitochondrial quality control in the pathogenesis of neurodegenerative diseases. For example, perturbation of mitophagy (mitochondrial degradation by autophagy) promotes the accumulation of the damaged mitochondria, a main source of reactive oxygen species. Mitochondria are highly dynamic organelles, and their quality is controlled by not only mitophagy but also mitochondrial dynamics (fission and fusion). Mitochondrial dynamics is crucial for mitochondrial renewal, distribution and proliferation, however, its regulatory mechanism in neurodegeneration remains poorly understood. Recently, we identified a novel 13-kDa protein (p13) whose expression is decreased in the pancreatic islets with oxidative stress. Further studies have revealed that p13 is also expressed in several brain regions, and affects mitochondrial dynamics by inhibiting their fusion process. We will present our data indicating the relation between p13 and Parkinson's disease, and propose that further functional analysis of p13 contribute to mitochondrial dynamics-based drug discovery for neurodegenerative diseases.

1-S-13-2 Inhibition of Parkin-opposing Deubiquitination Enzyme USP14 as a therapeutic approach to enhance clearance of misfolded proteins in PD

Elena Ziviani

Dept. Biol. Univ. Padua

USP14, mammalian homologue of fly gene CG5384, was identified following a Deubiquitination Enzyme (DUB)-based RNAi screening as a novel regulator of ubiquitination of mitochondrial Mitofusin (Mfn). Our data show that USP14 is opposing Parkin in the ubiquitination of Mfn and USP14 suppression is sufficient to promote mitophagy in the absence of Parkin, via stabilization of Mfn ubiquitinated forms. We propose that suppression of USP14 activity might be instrumental for the activation of mitochondria clearance pathway downstream of Parkin and can be beneficial in ameliorating locomotor deficits in animal models of PD.

1-S-13-3 Alpha-synuclein conformational states and their impact on vesicle trafficking

Luigi Bubacco

Dept. Biol. Univ. Padua

Alpha-synuclein (aS) is one of the emerging molecular players in synaptic function. This 140 aa protein localized at presynaptic terminals, and several studies are characterizing aS as protein that binds synaptic vesicle membranes and likely assists vesicle trafficking and SNARE complex formation. *Wild type* aS has also been reported to bind actin, slowing down its polymerization and accelerating its depolymerization, probably by sequestration of monomers that leads to an alteration of exo- and endocytic traffic. The peculiarity of aS is that simply an increase in its expression levels leads to abnormal aggregation and neuronal degeneration both in vitro and in vivo. A paradigm of this behaviour is found in multiplication mutations that increase expression levels by 50-100% and cause Parkinson's disease or dementia with Lewy bodies. To unravel its physiological role we started from a characterization of the tightly regulated equilibrium between water-soluble and membrane-bound and the posttranslational modifications that may control aS competence to interact with other cellular partners. To account for the Janus like quality of aS, the complex sequence of events that starts with aS aggregation and leads to its action as endotoxin will be discussed.

1-S-13-4 SUMOylation Pathways in Synaptic Development and Neurodegeneration

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Small ubiquitin-like modifier-1 (SUMO1) plays a number of roles in cellular events and recent evidence has given momentum for its contributions to neuronal development and neurological disorders. We have generated a SUMO1 transgenic mouse model with overexpression in neurons to identify in vivo conjugation targets and the functional consequences of their SUMOylation. Immunoprecipitation of SUMOylated proteins from total brain extract and proteomic analysis revealed a number of candidate proteins from a variety of functional classes, including a number of synaptic and cytoskeletal proteins. SUMO1 modification of synaptotagmin-1 was found to be elevated as compared to non-transgenic mice. This observation was associated with an age-dependent reduction in basal synaptic transmission and impaired presynaptic function as shown by altered paired pulse facilitation, as well as a decrease in spine density. The changes in neuronal function and morphology were also associated with a specific impairment in learning and memory while other behavioral features remained unchanged. These findings indicate a significant contribution of SUMO1 modification on neuronal function which has implications for mechanisms involved in mental retardation and neurodegeneration.

2-S-01-1 Significant Role of Pharmacology in Expected Outcomes from Open Innovation

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The recent advances of medicine and medical care, accompanied with development of high content technology and invention of revolutionary medical devices for diagnosis and treatment, have been achieved by progression of medical sciences including pharmacology. Above all, enhanced quality of medication is, in many cases, ascribed to discovery of innovative drugs, thus at least partly based on pharmacological efforts in open innovation, integrated and functional collaboration between academia and industry. In spite of these efforts, evaluating the general benefit of pharmacotherapy in treating patients, some diseases and symptoms, in contrast with hypertension, hyperlipidemia or hyperglycemia, are given limited benefits and still remained to treat enough in clinic. Such dissatisfaction of medicating the disease (for instance, stroke or diabetic complications) may be originated from inadequate or incomplete understanding of the nature of the disease. We therefore need to exactly understand its pathologic mechanism and to find adequate solution of the clinical problems. Then, we will discuss how to overcome the current situation and reach the goal to favorable medication through playing significant roles of pharmacology upon open innovation.

2-S-01-2 Translational Pharmacology and Zebrafish-Based Drug Discovery

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The next generation discipline of systems pharmacology aims to combine experimental analysis and computational modeling of in vivo networks with quantitative pharmacology approaches to drive the drug discovery processes, predict rare adverse events, and catalyze the practice of personalized medicine.Here, we introduce the strategy of zebrafish-based quantitative and systems pharmacology, which synergistically combine the desirable features of systems pharmacology and emerging technology of zebrafish-based screening system for functional omics and chemical biology. Zebrafish-based systems pharmacology that analyze in vivo regulatory networks involved in drug action can account for a drug's multiple targets and for the effects of genomic, epigenomic, and posttranslational changes on the drug efficacy. Zebrafish-based drug discovery has become a potential strategy by its high throughput quantitative in vivo screening and has already succeeded in several examples of phenotype-based drug discovery and personalized medicine.This new translational pharmacology can drive drug discovery and shape precision medicine.

2-S-01-3 AMED Strategy of Drug Discovery Support to Accelerate Delivery of New Medicines

Yoichi Kurebayashi

Japan Agency for Medical Research and Development

AMED Strategy of Drug Discovery Support to Accelerate Delivery of New Medicines
Japan's health and medical administration is facing challenges, such as changing medical needs due to rapidly aging society, growing trade deficit due to increasing foreign import of new drugs, and increasing global competition in Pharma research and development. In this context, Department of Innovative Drug Discovery and Development (iD3), Japan Agency for Medical Research and Develoment (AMED), has set broad, integrative strategies to boost, support and innovate drug discovery research in academic institutions and pharmaceutical industry. Drug Discovery Support Network is the core project of iD3 to accelerate translation of promissing basic researches into innovative new medicines. In this presentation, recent development and challenges of the Drug Discovery Support Network project will be outlined with other other new initiatives that iD3 has started and pursued to date.

2-S-01-4 Drug Discovery in Academia–from the drug screening with new ideas to the clinical trial in our university hospital—

Masatoshi Hagiwara

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Patients of congenital diseases have abnormalities in their chromosomes and/or genes. Therefore, it has been considered that drug treatments can serve to do little for these patients more than to patch over each symptom temporarily when it arises. Although we cannot normalize their chromosomes and genes with chemical drugs, we may be able to manipulate the amounts and patterns of mRNAs transcribed from patients DNAs with small chemicals. Based on this simple idea, we have looked for chemical compounds which can be applicable for congenital diseases and found INDY, TG003, and SRPIN340, and RECTAS. These are promising as clinical drugs for Down syndrome, Duchenne muscular dystrophy, Denys Drash Syndrome, and Familial dysautonomia, respectively. Through the development of these chemicals, we eventually succeeded to find candidate compounds available for more common diseases such as viral infections, cancers, age-related macular degeneration, chronic pain, and Parkinson disease. The preparation of the clinical trial of the ant-virus drug is under the way in our university hospital as an "Academia Drug". We propose the strategy to maximize the serendipity for the academic drug discovery with open innovation.

2-S-02-1 Overview

Yoshikatsu Kanai

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SGLT2 inhibitors have been developed as anti-diabetic drugs. They target Na+/glucose cotransporter SGLT2 in the renal proximal tubules responsible for the reabsorption of glomerular filtered glucose. By inhibiting SGLT2, SGLT2 inhibitors increase glucose excretion into urine and decrease blood glucose level in an insulin-independent manner in patients with type-2 diabetes. By improving blood glucose level, SGLT2 inhibitors ameliorate the symptoms and pathological changes associated with diabetes. SGLT2 inhibitors dramatically alter systemic glucose homeostasis because SGLT2 mediates massive glucose reabsorption. Renal tubular reabsorptions, in general, by preventing urinary loss of low molecular weight hydrophilic compounds and inorganic ions, play critical roles in the systemic homeostasis. Therefore, such transporters can be the targets of drugs to recover homeostasis disturbed in disease state. In this context, diuretics inhibiting renal electrolyte transporters and uricosuric drugs inhibiting urate reabsorption transporters have been used clinically. This idea can be extended to developing new categories of drugs to inhibit renal tubular transporters to recover homeostasis in diseases.

2-S-02-2 Effects of SGLT2 inhibitors on blood pressure and renal function

Akira Nishiyama

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Accumulating clinical evidence has revealed that treatment with SGLT2 inhibitors elicits a significant blood pressure reduction. However, the precise mechanism responsible for the SGLT2 inhibitor-induced blood pressure reduction is unclear. We have shown that treatment with selective SGLT2 inhibitors significantly decreased blood pressure with normalization of its dipping pattern in salt-treated obese rats and metabolic syndrome rats. During treatment with SGLT2 inhibitors, blood pressure reduction was associated with an increase in urinary excretion rate of sodium in these animals. Interestingly, urinary osmolality was consistently decreased by treatment with SGLT2 inhibitors. Furthermore, SGLT2 inhibitors-induced natriuresis was not enhanced by the addition of hydrochlorothiazide or hydrochlorothiazide+furosemide. These data suggest that SGLT2 inhibitors elicit an anti-hypertensive effect with improvement of dipping pattern of blood pressure through natriuresis in subjects with obese or metabolic syndrome. Furthermore, SGLT2 inhibitors-induced natriuresis cannot be solely explained by osmotic diuresis. Further studies are under way to examine the effects of SGLT2 inhibitor on the regulation of renal sodium transports.

2-S-02-3 Mechanism of SGLT2 Inhibitor-induced Decrease in Serum Urate Level

Ikumi Tamai

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SGLT2 inhibitors commonly lower serum urate (SUA) level. In clinical study, luseoglifrozin increased urinary excretion of urate (UEua) and the extent of increase of UEua mostly explained the decrease of SUA level. In addition, the increase in UEua was correlated with an increase in urinary D-glucose excretion, but not with the plasma luseogliflozin concentration. Furthermore, luseogliflozin had no direct effect on renal urate transporters at clinically relevant concentration. Accordingly, SUA-lowering effect was considered as the uricosuric effect which is common to SGLT2 inhibitors and is associated with the glycosuria. GLUT9 isoform 2 (SLC2A9b) is expressed at the apical membrane of the kidney tubular cells and transports both urate and D-glucose. By in vitro transporter-expressing cells, we observed trans-stimulation of urate efflux by 10 mM D-glucose, a high concentration of glucose that existed under SGLT2 inhibition. In addition, uptake of urate by GLUT9 isoform 2 was cis-inhibited by 100 mM D-glucose, a concentration assumed to exist in collecting ducts. Accordingly, it was demonstrated that UEua could be increased by SGLT2 inhibitor-induced glycosuria with alterations of urate excretory transport activity. Reference : Biopharm Drug Dispos 35 : 391-404 (2014).

2-S-02-4 A renal tubular transporter mediating reabsorption of β-hydroxybutyrate: a potential target of drugs increasing urinary ketone body excretion

Shushi Nagamori

Dept. Bio-sys Pharmacol., Osaka Univ. Grad. Sch. Med.

Ketone bodies are the important energy source particularly when glucose is starved. Because they are filtered by glomerulus in kidney, proximal tubules reabsorb them to recover in blood. In diabetic condition, ketone body production is increased so that its blood level is elevated, which causes diabetic ketoacidosis. Although Na^+-dependent monocaeboxylate transporters and H^+-coupled monocaeboxylate transporters have been proposed to mediate the reabsorption of a ketone body β-hydroxybutyrate (β-HB), their contribution to renal ketone body reabsorption has not been estimated. By means of metabolomics to compare the urine of the OATN1-knockout mice and that of wild type mice, we have found that an orphan transporter OATN1 mediates the reabsorption of β-HB. In alloxan-induced diabetic mice in which blood β-HB is increased, we have confirmed that OATN1 homo-knockout mice exhibit much higher renal β-HB excretion compared with wild type mice. Furthermore, the inhibitors of OATN1 largely reduced the blood β-HB level, suggesting that OATN 1 can be a target of drugs to increase urinary β-HB excretion and decrease blood β-HB level, which may be beneficial for the treatment of diabetic ketoacidosis.

2-S-03-1 Magic bullets for parasitic diseases: drugs from Japan

Kiyoshi Kita

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Parasitologists in Japan have been trying to discover new means to combat parasites and making significant contributions towards developing tools for global parasitic disease control. In the early 1970s, *Streptomyces avermectinius*, the source of ivermectin, was discovered by Prof. Satoshi Omura, who is a winner of Nobel Prize 2015, and renewed and vigorous screening of microbial metabolites in recent years is discovering new antiprotozoals and anthelminthics. Many people in developing countries still suffer from parasitic infectious diseases while, in developed countries, especially those caused by opportunistic infection due to the use of immunosuppressants and HIV/AIDS are increasing. In addition, mergence of strains resistant to the current front-line drugs cannot be disregarded. Consequently, through the new knowledge and understanding emanating from basic research into parasitic adaptation and its development for clinical application, Japanese scientists are retaining a significant role at the forefront of the control of emerging and re-emerging parasitic diseases. In this presentation, past, now and future of the discovery of the anti-parasites drugs will be discussed. These include ivermectin, nafuredin, atpenin A5, flutolanil and ascofuranone.

2-S-03-2 Design of treatments for cutaneous and visceral leishmaniasis

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Leishmaniasis is a disease complex, with cutaneous (CL) and visceral (VL) manifestations, caused by parasitic protozoa that survive and multiply in host macrophages. The current treatments for both forms of the disease are limited by cost, toxicities, and variable efficacy. For CL, where there are few acceptable treatments, variation in Leishmania species drug sensitivity and pathology contributes to this variation whereas for VL it is not understood why treatments that are effective in India are significantly less so in East Africa.
We are using mouse models of infection of both CL, caused by Leishmania major, and VL, caused by Leishmania donovani, to explore novel formulations, both topical and nanoparticle, to improve drug delivery and drug efficacy. The effect of the disease status on drug distribution, drug disposition and pharmacokinetics has been determined. In the mouse VL model, PK PD analysis has helped determine dose and time dependency of amphotericin B liposomes and miltefosine. The data will be discussed in relation to design of improved treatments.

2-S-03-3 Research & Development and Distribution of medicines for eliminating Tropical Diseases: Introduction of our activities as an example of the approach by pharmaceutical industries

Makoto Asada

CINO, PC HQ, Eisai Co., Ltd.

Pharmaceutical industries are expected to play a crucial role in combatting Neglected Tropical Diseases (NTDs) affecting low-income countries by providing effective medicines to patients in easy-to-access way. However in order to realize this mission, various challenges ever unexperienced within the industry are required to be overcome. For examples, aside from the issue of cost and benefit balance, knowledge and research tools of tropical diseases, clinical study networks, regulatory experiences and distribution logistics in the endemic countries are all sparse in existing pharma industries. Recently, various international approaches for eliminating tropical diseases have been initiated, which encourage and support the activities for drug development to those diseases by combining expertise of academia, PDPs (product development partners) and industries. In this presentation, our experiences on such collaboration activities for drug development with DNDi (Drugs for Neglected Diseases initiative) and other PDPs/academia will be introduced. Also, our efforts for enhancing access to medicines for patients in developing countries will be touched.

2-S-03-4 Japan's Global Health R&D Leadership

Kei Katsuno

Global Health Innovative Technology Fund

The first of its kind in Japan, the Global Health Innovative Technology Fund (GHIT Fund) is a public–private partnership between seven Japanese pharmaceutical companies, the Japanese Government, the Bill & Melinda Gates Foundation, the Wellcome Trust, and UNDP. Launched in April 2013 with an initial commitment of more than US$100 million, the organization taps Japanese research and development (R&D) to fight neglected diseases. The GHIT Fund invests and manages a portfolio of development partnerships aimed at neglected diseases that afflict the world's poorest people. GHIT mobilizes Japanese pharmaceutical companies and academic and research organizations to engage in the effort to get new medicines and vaccines to people who need them most, with Japan quickly becoming a game-changer in global health. In just two and a half years since it was formed, GHIT has invested in the development of more than 40 new products, with allocations totaling more than US $50 million. As of 2015, GHIT is advancing six clinical trials in Burkina Faso, Ivory Coast, Tanzania, Uganda, Thailand, Peru, and Bolivia, and two more clinical trials will begin in 2016.

2-S-04-1 HSPs as potential therapeutic targets in SCA14 associated with amyloidogenic assembly of mutant PKCγ

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Amyloid assemblies are associated with a wide range of human neurological disorders, including Alzheimer's and Parkinson's diseases. Here, we identify protein kinase Cγ (PKCγ), a serine/threonine kinase mutated in the neurodegenerative disease spinocerebellar ataxia type 14 (SCA14), as a novel amyloidogenic protein. We found that overexpression of PKCγ in cultured cells, as well as in vitro incubation of purified PKCγ without heat or chemical denaturants, result in amyloid-like PKCγ fibril formation. We also observed that SCA14-associated mutations in PKCγ greatly enhanced amyloid-like fibril formation both in cultured cells and in vitro. Furthermore, time-lapse microscopic imaging in cultured neuronal cells demonstrated that aggregates of mutant PKCγ are highly toxic. In these cells, aggregates are co-localized with several heat shock proteins (HSPs), which physically interact with mutant PKCγ. Overexpression of HSP70 and HSP 40 reduced aggregate formation and cytotoxicity of mutant PKCγ in cultured cells. These findings demonstrate that HSPs act directly to regulate amyloid-like PKCγ fibril formation, and thus identify HSPs as potential therapeutic targets in SCA14.

2-S-04-2 The involvement of NOX1/NADPH oxidase in the emotional behavior

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Reactive oxygen species (ROS) have been implicated in the development of psychiatric disorders. We presently report NOX1/NADPH oxidase is the source of ROS that regulate the emotional behavior demonstrated in murine models. When anxiety-like behaviors were evaluated, wild-type mice (WT) and mice deficient in *Nox1* (NOX1-KO) showed similar anxiety levels. However, increased anxiety-like behaviors in WT following acute restraint stress were markedly ameliorated in NOX1-KO. Similarly, reduced sucrose preference demonstrated in WT chronically administered with corticosterone (CORT) was significantly suppressed in NOX1-KO. In WT treated with CORT, the levels of brain-derived neurotrophic factor (BDNF) mRNA and of transcripts containing exon IV of *BDNF* gene were significantly decreased, but they were sustained in NOX1-KO. ROS production was significantly elevated in the prefrontal cortex (PFC) of mice administrated with CORT, while the level of NOX1 mRNA was up-regulated in the ventral tegmental area (VTA), not in PFC. Finally, selective silencing of NOX1 in VTA by AAVrh10 virus expressing miRNA restored CORT-induced depressive-like behaviors and reduced expression of BDNF mRNA. ROS derived from NOX1 may therefore play a key role in the emotional behavior induced by stress.

2-S-04-3 Molecular mechanisms of a familial epilepsy caused by LGI1 mutations

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Mutations of LGI1, a neuronal secreted protein, are linked to autosomal dominant lateral temporal lobe epilepsy (ADLTE). Also, anti-LGI1 autoantibodies are frequently detected in patients with limbic encephalitis characterized by seizures and memory impairment. We have reported that LGI1 functions as a ligand for an epilepsy-related transmembrane protein, ADAM22; LGI1 enhances AMPA-type glutamate receptor-mediated synaptic transmission; and loss of LGI1 in mice reduces AMPA receptor-mediated synaptic transmission and causes lethal epilepsy. However, the patho-physiological functions of LGI1 still remain unclear. Here, we classified LGI1 missense ADLTE mutations as either secretion-defective or -competent, and generated two mouse models of ADLTE harboring two representative LGI1 mutations. Both mutations reduced synaptic LGI1-ADAM22 interaction in the mouse brain due to protein conformational defects. A chemical chaperone restored the folding of the secretion-deficient mutant protein and its binding to ADAM22 and ameliorated the increased seizure susceptibility of the mutant mice. This study establishes an essential role of the LGI1-ADAM22 interaction to regulate the brain excitability (Yokoi et al. Nat. Med. 2015).

2-S-04-4 The contribution of adult born neurons in memory consolidation during wake sleep cycles

Masanori Sakaguchi, Deependra Kumar, Masanobu Hayashi, XiFang Hayashi, Gopakumar Changarathil, Takeshi Sakurai

Sleep institute, Univ. Tsukuba

The process which affects memory consolidation during wake sleep cycles has been unknown. We found that optogenetically silencing adult-born neurons in the mice dentate gyrus during specific wake sleep cycle impairs memory consolidation. The adult born neurons' contribution to this process depends on the maturation status of the neurons and the nature of the memory. Furthermore, brain activity imaging revealed that adult-born neurons impact the hippocampal activity of memory consolidation during sleep. These results suggest that intervening specific neuronal circuits during each wake-sleep period could uncover its unique function.

2-S-05-1 Activity-dependent bidirectional regulation of terminal neuronal maturation in the adult hippocampus

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Defects in the terminal maturation of central neurons have been implicated in the pathophysiological basis of neuropsychiatric disorders. However, the regulatory mechanism of the terminal maturation remains largely unknown. Here we show that neuronal activity bidirectionally regulates the terminal maturation of adult mouse hippocampal granule neurons. Neuronal excitation by electroconvulsive stimulation (ECS) rapidly reduced expression of molecular markers for mature granule neurons and induced immature neuron-like functional properties such as higher excitability. The phenotypes of ECS-treated neurons were maintained for more than 2 weeks after repeated ECS. Block of NMDA receptors after the period of ECS counteracted maintenance of the immature phenotypes and promoted rematuration, suggesting that the enhanced excitability of ECS-treated neurons autonomously supports their immature state. These results demonstrate that the terminal maturation of granule neurons is reversed and advanced by excitation and inhibition, respectively. We propose that cell stage-specific regulation of neuronal maturation by activity would be a novel strategy for treating neuropsychiatric disorders via rescue of the defective neuronal maturation.

2-S-05-2 An in vitro model for studying repetition-dependent memory consolidation; Long-lasting synaptic enhancement in cultured hippocampal slices and its suppression by a stress mimicry.

Keiko Tominaga-Yoshino, Shinichi Saito, Akihiko Ogura

Osaka Univ. Grad. Sch. Front. Biosci.

We previously reported that the repeated inductions of chemically induced LTP produced a long-lasting enhancement of synaptic strength in cultured hippocampal slices. Naming this phenomenon RISE (Repetitive-LTP-Induced Synaptic Enhancement), we assume this as an in vitro reproduction of repetition-dependent memory consolidation since it is long-lasting, specific to input pathway and accompanied by new synapse formation. RISE develops through stochastic processes ; raised fluctuation of synapses (both generation and retraction are increased) followed by biased fluctuation (retraction returns to basal level earlier than generation). It is shown that BDNF is involved in RISE production and that a transient expression of Ca^{2+}-permeable AMPA receptors is critical. If RISE is the reproduction of memory consolidation, RISE should also be affected by stress. Dexamethasone (Dex), a stress mimicry, applied 12h after a RISE-producing stimulus blocked the development of RISE. However, Dex was ineffective when applied several days after the stimulus. RISE should serve as a model for analyzing the cellular mechanisms underlying stress-induced memory defects.

2-S-05-3 Novel cognitive mechanism by calcium/calmodulin-dependent protein kinase II activation

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Calcium/calmodulin-dependent protein kinase II (CaMKII) is highly localized in the post-synaptic densities of excitatory synapses and becomes constitutively active through autophosphorylation following hippocampal LTP (Fukunaga et al., 1993). CaMKII facilitates synaptic efficacy through direct phosphorylation of post-synaptic AMPA receptor subunit GluA1 (Ser-831). Recently, we focus on CaMKII activation for cognitive enhancement by Na^+/Ca^{2+} exchangers (NCXs). NCXs have three different isoforms (NCX1, NCX2, NCX3) encoded by distinct genes. We also found that mice lacking NCX 2 (NCX2-KO) exhibit impaired memory. NCX2-KO mice showed significantly impairment of learning and memory-related behaviors and hippocampal LTP. CaMKII autophosphorylation significantly decreased in the hippocampal CA1 region of NCX2-KO mice compared to wild-type mice. The enhancement of CaMKII autophosphorylation following LTP was also impaired in NCX2-KO mice. Furthermore, the decreased CaMKII autophosphorylation was closely associated with the reduced GluA1 (Ser-831) phosphorylation in the hippocampus. Taken together, the decreased CaMKII activity with concomitant LTP impairment likely accounts for the learning disability observed in NCX2-KO mice.

2-S-05-4 Molecular Mechanisms and Effects of Aging on Rewriting of Motor Learning and Cerebellar Plasticity

Sho Kakizawa

Dept. Biol Chem., Kyoto Univ. Grad. Sch. Pharmaceu. Sci.

Rewriting of memory is essential for adaptation to new environment. However, mechanisms of memory rewriting have been largely unknown. Eyeblink conditioning, cerebellar-dependent motor learning, has acquisition and rewriting (extinction) phases. Although involvement of LTD at parallel fiber (PF) synapse in acquisition process is well known, mechanisms of extinction have yet to be examined.

Because LTP at PF synapse (PF-LTP) is known to reverse the saturation of PF-LTD, involvement of PF-LTP in the extinction of eyeblink conditioning is hypothesized. PF-LTP is also indicated to be induced by NO-induced Ca^{2+} release (NICR), a novel Ca^{2+} release mechanism, in PCs. Because NICR is induced by S-nitrosylation of C3636 in ryanodine receptor 1 (RyR1), a Ca^{2+}-release channel, by NO, knockin mice expressing RyR1 carrying C3636A mutation ($RyR1^{C3636A}$ mice) was generated. In $RyR1^{C3636A}$ mice, as well as NICR and PF-LTP, extinction but not acquisition of eyeblink conditioning was impaired. Therefore, NICR and PF-LTP were suggested as molecular and cellular basis for extinction of eyeblink conditioning.

Another topic on memory rewriting is effect of aging. Effects of aging on NICR and PF-LTP were also investigated, and will be shown in my talk.

2-S-06-1 Adenovirus-Mediated Overexpression of TNNI3K Promotes Cardiomyocyte Hypertrophy

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Cardiac troponin I-interacting kinase (TNNI3K) is a novel cardiac-specific kinase gene. The aim of the present study was to investigate the effects of TNNI3K on neonate rat cardiomyocyte hypertrophy. Cardiomyocytes were infected with either a control adenovirus carrying green fluorescent protein (Ad-GFP) or Ad-TNNI3K. Compared with Ad-GFP, the Ad-TNNI3K could increase in sarcomere organization, cell surface area, ^{3}H-leucine incorporation and ν-MHC re-expression. This type of hypertrophic phenomenon is similar to the observation in cardiomyocytes induced by ET-1. The cells were further infected with Ad-GFP or Ad-TNNI3K in ET-1-induced hypertrophic, Ad-TNNI3K could also increase in sarcomere organization, cell surface area and ^{3}H-leucine incorporation.The results suggest that cardiomyocytes hypertrophy could be induced and accelerated by TNNI 3K overexpression. Therefore, TNNI3K might be an interesting target for the clinical treatment of hypertrophy.

2-S-06-2 Evidence, hypotheses and significance of MAP kinase TNNI3K interacting with its partners

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TNNI3K plays important roles in promoting cardiac differentiation, maintaining beating rhythm and contractual force. The molecular structure of TNNI3K contains three kinds of domains : a seven or ten NH_2-terminal ankyrin repeat domain followed by a protein kinase domain and a COOH-terminal serine-rich domain. There are many binding sites in the structure of TNNI3K for binding to ATP, magnesium, nucleotide, protein kinase C, antioxidant protein-1 and cTnI. This review summarizes the evidence, hypothesis and significance of TNNI3K interacting with TNNI3 and its other putative interaction partners. From the literature, the interaction partners of TNNI3K are divided into 2 types : to increase or suppress cardiac performances. Following their binding sites, they are divided into other 2 types : binding to C-terminal domain or binding to both ankyrin repeat domain and C-terminal domains. To date, a well understood partner of TNNI3K is cTnI, from the molecular structure, physiological function, mechanisms and its significances in physiological and pathophysiological conditions. There are many reasons to believe that, with more understanding on the TNNI3K interacting with its partners, we can understand more roles of TNNI3K in some cardiac diseases.

2-S-06-3 Circulating TNNI3K levels are useful diagnosis biomarker for ischemic myocardial diseases

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Availability of circulating TNNI3K level was investigated as a new diagnosing biomarker for acuate ischemic cardiac diseases by using anti-TNNI3K poly or monoclonal antibodies in patients diagnosed as AMI, chronic heart failure, acute renal failure ; and in healthy volunteers groups. Our data showed that circulating TNNI3K levels were significantly higher in AMI when compared with other two groups ($p<0.001$), indicating that measurement of circulating TNNI3K is a novel and useful diagnosing tool for ischemic heart diseases including AMI. Our data also showed that the sensitivity and specificity are higher, ROC curve is best than that of polyclonal antibodies, indicating that measurement of circulating TNNI 3K level using the monoclonal-antibodies may be a novel and useful tool to diagnose AMI. In conclusion, using TNNI3K as a molecular target is a new potential approach for developing diagnostic agents for ischemic cardiac diseases.

2-S-06-4 TNNI3K affects the cardiac contractile function by cTnI phosphorylation

Li Song

Cardiovascular Institute & Fu Wai Hospital, Chinese Academy of Medical Sciences

TNNI3K is a novel cardiac-specific functional kinase that could bind to cTnI in a yeast two-hybrid screen. The phosphorylation of cardiac troponin I (cTnI) plays an important role in the contractile dysfunction associated with heart failure. The purpose of this study was to investigate whether TNNI3K could phosphorylate cTnI and the effect in cardiac contractile function. The study first confirmed that TNNI3K could interact with cTnI by Co-Immunoprecipitation, and directly phosphorylated Ser43 and Thr143 in vitro. Furthermore, enhanced phosphorylation of cTnI correlated with rTNNI3K (rat TNNI3K) overexpression, and phosphorylation was reduced when rTNNI3K was knocked down in adult rat cardiomyocytes. Finally, the contraction of cardiomyocytes, modulated by TNNI3K-mediated phosphorylation of cTnI, increased with rTNNI3K overexpression and decreased with rTNNI3K knockdown in adult rat ventricular myocytes. In summary, TNNI3K may be a novel mediator of cTnI phosphorylation and contribute to the regulation of cardiac myofilament contraction function.

2-S-06-5 Dystrophin-Deficient Cardiomyocytes Derived From Human Urine: New Biologic Reagents for Drug Discovery

Yuanyuan Zhang

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The ability to extract somatic cells from a patient and reprogram them to pluripotency opens up new possibilities for personalized medicine. Induced pluripotent stem cells (iPSCs) have been employed to generate beating cardiomyocytes from a patient's skin or blood cells. Urine was chosen as a starting material because it contains adult stem cells called urine-derived stem cells (USCs). USCs express the canonical reprogramming factors c-myc and klf4, and possess high telomerase activity. Pluripotency of urine-derived iPSC clones was confirmed by immunocytochemistry, RT-PCR and teratoma formation. Urine-derived iPSC clones generated from healthy volunteers and a DMD patient were differentiated into beating cardiomyocytes using a series of small molecules in monolayer culture. Results indicate that cardiomyocytes retain the DMD patient's dystrophin mutation. Physiological assays suggest that dystrophin-deficient cardiomyocytes possess phenotypic differences from normal cardiomyocytes. Results demonstrate the feasibility of generating cardiomyocytes from a urine sample and that urine-derived cardiomyocytes retain characteristic features that might be further exploited for mechanistic studies and drug discovery.

2-S-07-1 Immunothrombosis in septic ARDS

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The pathogenesis of sepsis includes the disturbance of blood-vascular homeostasis, which may cause multiple organ failure, circulatory shock, and DIC, leading to high mortality. Neutrophil-associated inflammation or microthrombus formation (Immunothrombosis) in the lung resulted in ARDS, the most important cause of death in sepsis. Histidine rich glycoprotein (HRG) is an 80 kDa glycoprotein with high histidine contents. HRG is known as the plasma factor to regulate coagulation/fibrinolysis, immune response and angiogenesis. Our recent studies showed that plasma HRG levels significantly decreased in CLP septic mice model and administration of HRG dramatically improved the survival rate of CLP mice associated with the inhibition of immunothrombosis and neutrophil extracellular trap (NET) formation in pulmonary vasculatures by keeping neutrophils quiescent morphologically and functionally. Supplementary therapy with HRG may provide a novel strategy for the treatment of septic patients by inhibiting immunothrombosis in blood vessels.

2-S-07-2 Uncontrolled immunothrombosis as a basis of sepsis-associated DIC

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Microvascular thrombosis is commonly considered to be harmful because it compromises the blood supply to organs. However, recent studies have suggested that microvascular thrombosis might play a role in the early defense against invading pathogens. This defensive role of thrombosis during infection is now referred to as immunothrombosis. Detection of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) triggers tissue factor expression on monocytes and neutrophil extracellular trap (NET) release by neutrophils, promoting immunothrombosis. Sepsis-associated disseminated intravascular coagulation (DIC) is considered to be an uncontrolled state of immunothrombosis, where the immune system is no longer able to restrict spreading of pathogens, inflammation, and coagulation. In this state, widespread microvascular thrombosis is detrimental because it causes ischemic damage to multiple organs.

Recombinant thrombomodulin (rTM) is a therapeutic option for the treatment of sepsis-associated DIC in Japan. In addition to its anticoagulant effect, rTM has the ability to dampen inflammation, in part through the sequestration of PAMPs and DAMPs. Thus, rTM may be useful for resolving PAMPs- and DAMPs-mediated DIC.

2-S-07-3 Hemin-induced NET formation and the effects of iron chelators on neutrophil functions

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Transfusion-related acute lung injury is serious adverse effect of transfusion. Although antibodies against leukocytes are considered as major inducers of excessive neutrophil activation, red cell concentrate (RCC) is the main cause in Japan. Then we have checked the effects of hemolysis using hemin (ferri-protoprphyrin IX).
Hemin increased CD11b expression, reactive oxygen species (ROS) production, and induced surface minute process formation within 15min. The possibilities of the morphological changes relating to neutrophil extracellular trap (NET) formation has been studied. Sytox green method, immunofluorescence using anti-histone or myeloperoxidase, and electron microscopy revealed that hemin induced NETs. These results suggested that hemin might be an inducer of acute lung injury (ALI). RCC especially stored for long period contained substantial concentration of hemin.
Deferasirox (DFS), new oral iron chelator, was reported to suppress NF-κB activation. Then we have checked the effects of DFS on NET formation. DFS was shown to decrease ROS production induced by PMA or fMLP, and to inhibit NET formation.
These results suggest the possible preventive effects of DFS on over-activation of neutrophil and relating pathophysiologies.

2-S-08-1 Prevention of diabetes using curcumin via stimulation of gut hormone secretion

Takanori Tsuda

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Glucagon-like peptide-1 (GLP-1) is one of the incretins and secreted from enteroendocrine L cells, which are present in the lower small intestine and large intestine. Enhancing GLP-1 action induces glucose-dependent insulin secretion. The preferable way for dietary factors to elevate GLP-1 actions is by stimulating the endogenous secretion of GLP-1. Therefore, stimulation of GLP-1 secretion is recognized as potential therapeutic targets in the prevention and treatment of type2 diabetes. This presentation provides information as to how dietary factors increase GLP-1 secretion. Concretely, we present our findings on the GLP-1 secretion-stimulating functions of curcumin. Curcumin significantly stimulated secretion of GLP-1 in vitro and in vivo. Our examination of structure-function relationships found that a β-diketone structure and at least one methoxy group on the aromatic ring are necessary for a curcumin derivative to promote GLP-1 secretion. The mechanism of action of by which curcumin stimulates GLP-1 secretion is increase in intracellular calcium and activation of Ca^{2+}/calmodulin-dependent kinase II. These findings provide a novel biological function of curcumin in regards to GLP-1 secretion.

2-S-08-2 The effectiveness of the curcumin on periodontal disease.

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Periodontitis is an infectious disease caused by periodontopathic bacteria. Cytokines involved in the inflammatory response activate osteoclasts, resulting in bone resorption. Conventional periodontal treatment including plaque control aims at removing these pathogens. Recently, controlling inflammation is important to reduce the risk of the periodontal tissue destruction. It is known that curcumin possesses an anti-inflammatory effect and its effectiveness on periodontitis has been clarified in animal and human experiments. In this presentation, the effectiveness of the curcumin on periodontitis will be discussed.

2-S-08-3 Translational Research of Highly Absorbable Curcumin for Heart Failure Therapy

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Heart failure is the final stage of cardiovascular diseases. It is necessary to find out the further medical therapy for heart failure. We previously reported that cardiac p300 HAT activity is required for the development of heart failure and curcumin, a natural p300-specific HAT inhibitor, has a therapeutic potency for heart failure. However, it is insufficient to exert its bioactivities due to its low solubility and poor gastrointestinal absorption. We have produced highly absorbable curcumin preparation, Theracurmin. In this session, we introduce our recent study using Theracurmin and summarize the translational research of Theracurmin for heart failure therapy.

2-S-08-4 Enhanced anti-tumor effects of the PD-1/PD-L1 blockade by combining a highly absorptive form of NF-κB/STAT3 inhibitor curcumin

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The PD-1/PD-L1 blockade is now recognized as one of the effective standard therapy for some human cancers such as melanoma and lung cancers, however clinical effect is still limited because of tumor-induced immunosuppression. Curcumin has been reported to inhibit NF-kB and STAT3 signals which have important roles for inducing immunosuppression. Here, we show not only that curcumin augments induction of tumor antigen specific T cells via acting on both cancer cells and immune cells, but also that combination of curcumin and local PD-1/PD-L1 blockade have synergistic anti-tumor activity. These results indicated that curcumin enhanced effect of PD-1/PD-L1 blockade and is an attractive strategy for development of effective combination cancer immunotherapy.

2-S-09-1 Synergistic regulation of biological molecules by the drugs for COPD

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COPD is the fourth leading cause of death worldwide and expected to become third in 2030. Anti-inflammatory drugs, such as steroids and phosphoriesterase 4 (PDE4) inhibitor, have been approved for COPD. However, those efficacies have been hampered by the development of adverse effects and tolerance, and the underlying mechanism remains unclear. Here we found that the synergistic regulation of IRAK-M and PDE4B2 by those drugs. Our findings may lead to further development of anti-inflammatory therapeutic strategies for COPD.

2-S-09-2 The role of myeloid-derived suppressor cells in chronic airway inflammation

Ichiro Horie, Yoichiro Isohama

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Myeloid-derived suppressor cells (MDSC), which are defined as $CD11b^+$ and $Gr\text{-}1^+$ cells in mice, can directly suppress T cells and macrophages, and negatively regulate immune responses. MDSC contribute to tumor immunity, chronic inflammation and autoimmune diseases, MDSCs may be a new therapeutic target for these diseases. In this present study, we examined whether MDSC contribute to chronic inflammation in airway, such as asthma and chronic obstructive pulmonary disease (COPD). First, we prepared asthma model that BALB/c mice were sensitized and challenged with ovalbumin (OVA). In OVA-sensitized mouse, MDSC increased in peripheral blood, spleen and lung, according to progress of pathology of airway inflammation. Interestingly, anti-Gr-1 antibody, a typical inhibitor of MDSC, exacerbated inflammatory pathology such as infiltrated cells and protein in bronchoalveolar lavage fluid (BALF), suggesting that MDSC play a protective role for chronic inflammation. In addition, we found that dexamethasone, one of glucocorticoids, increased MDSC in vitro, suggesting that glucocorticoid may exert anti-inflammatory effect via up-regulation of MDSC. Thus, our data implicates that MDSC may be a therapeutic target in chronic airway inflammation such as asthma and COPD.

2-S-09-3 Abnormal expression of zinc transporter ZIP2 in a novel mouse model of obstructive lung diseases

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Zinc ion (Zn^{2+}) is an essential dietary metal ion that has pleiotropic effects in many tissues including airway and lung. Patients with obstructive lung diseases like cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD), refractory lung diseases characterized by mucus hypersecretory and inflammatory phenotypes, have a defect in the regulation of intracellular Zn^{2+} level. Here, we identified in a CF/COPD murine model (βENaC-Tg mice) that expression of a novel splicing isoform of zinc transporter ZIP2 (ΔC-ZIP2) is increased in airway epithelial cells, which possibly results in the production of C-terminus-deleted immature ZIP2 protein. Importantly, increased expression of ΔC-ZIP2 transcript and inverse correlation between the expression of ΔC-ZIP2 transcript and WT-ZIP2 protein or intracellular Zn^{2+} level were observed in human CF and COPD-like airway epithelial cells. Moreover, depletion of intracellular Zn^{2+} with Zn^{2+} chelator up-regulated the gene expression of mucus and inflammatory cytokines in normal human airway epithelial cells. Thus, our finding demonstrates that a novel splicing switch of ZIP2 genes in airway epithelial cells possibly controls the pathology of obstructive lung diseases.

2-S-09-4 Development of the novel therapeutic drug for COPD

Michiko Horiguchi

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Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease characterized by cough, primarily dyspnea, sputum production, and other respiratory symptoms, causes widespread and irreversible alveoli collapse. Recent reports suggest that COPD mortality is increasing, and COPD is estimated to emerge as the third leading cause of death worldwide by WHO. However, a practically useful compound for regenerating pulmonary alveoli is yet to be reported. Regenerative medicine shows potential for addressing this intractable disease, but no regenerative treatments for human alveoli have been described to date. The aim of this study was to identify a treatment target molecule which is able to regenerate collapsed alveoli. Previously, we have elucidated that Akt phosphorylation is involved in elastase-induced COPD model mouse. In the present study, we directed our attention to phosphoinositide 3-kinase (PI3K)-Akt signaling and examined whether PI3K inhibitors display the pulmonary alveolus regeneration.

2-S-10-1 Regulation of a parasitic disease by host innate immunity

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p62 (also known as Sqstm1) is a selective autophagy adaptor with a ubiquitin-binding domain. However, the role of p62 in the host defense against Toxoplasma gondii infection is unclear. Here, we show that interferon γ (IFN-γ) stimulates ubiquitin and p62 recruitment to T. gondii parasitophorous vacuoles (PVs). Some essential autophagy-related proteins, but not all, are required for this recruitment. Regardless of normal IFN-γ-induced T. gondii clearance activity and ubiquitination, p62 deficiency in antigen-presenting cells (APCs) and mice diminishes the robust IFN-γ-primed activation of CD8+ T cells that recognize the T. gondii-derived antigen secreted into PVs. Because the expression of Atg3 and Irgm1/m3 in APCs is essential for PV disruption, ubiquitin and p62 recruitment, and vacuolar-antigen-specific CD8+ T cell activation, IFN-γ-mediated ubiquitination and the subsequent recruitment of p62 to T. gondii are specifically required for the acquired immune response after PV disruption by IFN-γ-inducible GTPases.

2-S-10-2 Mechanism of immunopathology during malaria: search for new drug targets

Cevayir Coban

IFReC, Osaka Univ.

Malaria caused by Plasmodium parasites is still one of the most devastating infectious diseases of the world, killing approximately 1 million people each year. Efforts to eliminate malaria have been compromised by the parasites' rapid evolution of drug resistance and the difficulty to develop effective vaccines. In my laboratory, we investigate the role of both innate and adaptive immunity in response to Plasmodium parasites and protective mechanism (s) elucidated by host. To do that, we have utilized several imaging technologies such as ultra-high field MRI and intra vital multi-photon microscopy to understand immunopathology caused by Plasmodium parasites. We hope to develop novel therapeutics in addition anti-malarials to rescue host from the consequences of immunopathology caused by parasites.

2-S-10-3 Potential of anti-influenza drug development targeting host nuclear network

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The influenza virus is a single-stranded RNA virus, and virus replication occur in the nucleus. Viral infection is associated with virus-driven hijacking of the host factors. Currently licensed anti-influenza drugs target viral NA proteins. The strains have recently emerged that show resistance to NA inhibitors. Thus, there is an urgent need to develop more effective antiviral therapeutics against influenza virus infection. Virus-host interaction could be novel targets for anti-influenza drugs. Using lipid library screen and lipidomics analysis we identified novel lipid mediators, which can suppress influenza virus replication via viral RNA nuclear export machinery, suggesting a link of host metabolic pathway to nuclear virus replication mechanism. In addition, a glowing body of work has shown that environmental stress can control chromatin structures that closely correlate with transcriptional regulation. We found that influenza virus disrupted stable chromatin organization, which controls virus replication mechanisms in the nucleus. These data suggest a crucial role of dynamic nuclear virus-host interaction in the pathogenesis of influenza virus infection. Those pathways could be potential targets for anti-influenza drug development.

2-S-10-4 Recognition of mycobacteria through C-type lectin receptors

Sho Yamasaki

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C-type lectin receptors (CLRs) comprise a large family of proteins that share a common structural motif and are involved in various immune responses. Among them, we found that several ITAM-coupled CLRs are pattern recognition receptors (PRRs) for mycobacteria. Characteristic glycolipids, glycoproteins and lipoglycans were identified in mycobacteria as ligands for these CLRs, such as Mincle, MCL and Dectin-2. These findings shed light on CLRs as emerging immune receptor family for wide spectrum of pathogens, particularly mycobacteria, and thus CLRs could be promising targets for the development of novel adjuvant. In this seminar, the recent progress and perspective on the function of CLRs will be discussed.

2-S-11-1 Cannabinoid functions in amygdala contribute to conditioned fear memory in diabetic mice

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The role of cannabinoid (CB) systems in conditioned fear memory was investigated in streptozotocin-induced diabetic mice (DM). The duration of freezing in DM was significantly longer than that in non-diabetic mice (NDM). The injection of WIN-55,212-2 after conditioning significantly prolonged the duration of freezing in NDM, but not in DM. The injection of CB1 receptor antagonist AM 251 after conditioning significantly shortened the duration of freezing in DM, but not in NDM. The injection of AM 251 before conditioning or before testing did not significantly affect the duration of freezing in DM. The protein levels of CB1 receptors, but not CB2 receptors and diacylglycerol lipase α, an endocannabinoid 2-arachidonoylglycerol synthase in amygdala were increased in DM. The injection of AM 251 into basolateral amygdala (BLA) significantly inhibited the duration of freezing in DM. The injection of AMPA receptor antagonist NBQX into BLA significantly inhibited the duration of freezing in DM. These results suggest that CB functions in amygdala are increased in DM and that enhanced glutamatergic function in the amygdala of DM activates the endocannabinoid system, which enhances fear memory via CB1 receptors.

2-S-11-2 Regulation of synaptic plasticity in the cortico-amygdala synapse by dietary polyunsaturated fatty acid balance

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Converging evidence suggests that a balance of omega 3 to omega 6 polyunsaturated fatty acid (PUFA) in the brain is involved in emotion, and that imbalance of these PUFAs contributes to mental illnesses, such as anxiety disorders. However, the underlying mechanism is unknown. We show that auditory fear memory, a type of emotional memory, and long-term potentiation (LTP) of synaptic transmission in the optogenetically isolated auditory cortico-amygdala synapse, which is one of the pathways transmitting conditioned stimulus in auditory fear conditioning, were both reduced in mice fed a high omega 3 to omega 6 PUFA ratio diet (3/6=0.97, a high 3/6 diet) compared with mice fed a diet with a lower ratio (3/6=0.14, a low 3/6 diet). These reductions were blocked by a cannabinoid CB1 receptor antagonist. The cholesterol content in brain tissues from mice fed a high 3/6 diet was lower than in brain tissues from mice fed a low 3/6 diet. The content of 2-arachidonoylglycerol did not differ in mice fed these diets. These findings suggest that PUFA balance-mediated alteration of fear memory is accompanied by modulation of LTP of neurotransmission in a synapse participating in fear memory.

2-S-11-3 Targeting the glutamatergic system to develop novel therapeutics for mood and anxiety disorders

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Dept. Neuropsychopharmacol., NIMH. NCNP.

Growing evidence indicates that glutamatergic neurotransmission plays important roles in the pathology and treatment of mood and anxiety disorders. A nonselective NMDA-R antagonist ketamine, which is approved as a dissociative anesthetic, has been shown to induce rapid antidepressant effects in patients and in rodent models. Riluzole, which is currently used to slow the progression of amyotrophic lateral sclerosis, is another candidate as an antidepressant or an anxiolytic drug. Riluzole has been shown to inhibit glutamate release and enhances glutamate uptake. We have previously reported that riluzole rapidly attenuated hyperemotional responses in olfactory bulbectomized rats, an animal model of mood and anxiety disorders. More recently, we demonstrated that riluzole shows clear anxiolytic-like effects in behavioral tests in rats. Drug approved for other uses has already passed a series of toxicity and other tests, its safety is known and the risk of failure in clinical trials are reduced. We believe that this strategy is practical to expedite the translation of preclinical findings of drugs with novel mechanisms of action such as glutamate transmission and related pathways into clinical applications.

2-S-11-4 Therapeutic potential of the intranasally administered GLP-2 derivative for the treatment of depression

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Glucagon-like peptide-2 (GLP-2) is derived from a proglucagon precursor in the gut and central nervous system (M.W. 3766.1), and used for the treatment of short bowel syndrome because of its potent intestinotrophic effect. Gs-coupled GLP-2 receptors in the brain are distributed in the dorsomedial hypothalamic nuclei, nucleus solitary tract, amygdala, thalamus, cerebellum, hippocampus, and cerebral cortex. In our previous studies, GLP-2 (i.c.v., mice) significantly reduced immobility time in the forced-swim test (FST) and tail suspension test, and also showed antidepressant-like effects in treatment-resistant depression model mice. In this symposium, we will give a talk on establishment of a new GLP-2 derivative for the delivery of GLP-2 to the brain by the intranasal administration and its antidepressant-like effects. A new nasal GLP-2 derivative was synthesized, and its brain permeability, stability, and antidepressant-like effects in the FST were observed. We have applied for a national patent and the patent cooperation treaty for the nasal GLP-2 derivative.

3-S-01-1 Cortical astrocytes rewire somatosensory synaptic circuits for neuropathic pain mechanical allodynia

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Although neuropathic pain such as mechanical allodynia is known to be induced by glial activation within the spinal cord, an effective treatment is still insufficient, indicating that novel therapeutic targets are critically needed. One such target may be the synaptic rewiring in the primary somatosensory (S1) cortex. However, its causal relationship to mechanical allodynia and its underlying cellular mechanisms remain unknown. Here, using in vivo two-photon microscopy imaging with genetic and pharmacological manipulations, we show that partial sciatic nerve ligation (PSL) injury elicits spontaneous somatic Ca2+ transients, thrombospondin-1 release in astrocytes, leading to synapse formation. Such activation of S1 astrocytes was evident only during a critical period (~1w post-injury), correlating with the temporal changes in S 1 extracellular glutamate levels and dendritic spine turnover following PSL injury. Blocking this astrocytic signaling pathway suppressed mechanical allodynia, while activating this pathway in the absence of injury induced long-lasting allodynia. Thus, these synaptogenic astrocytes are a key trigger for S1 synaptic circuit rewiring that causes neuropathic pain mechanical allodynia.

3-S-01-2 Observation and manipulation of glia assembly

Kenji Tanaka

Keio Univ. Sch. Med. Dept. Neuropsychiatry

The development of gene-encoded indicators and actuators to observe and manipulate cellular functions is being advanced and investigated. Expressing these probe molecules in glial cells is expected to enable observation and manipulation of glial cell activity, leading to elucidate the behaviors and causal roles of glial cells. The first step toward understanding glial cell functions is to express the probes in sufficient amounts, and the Knockin-mediated ENhanced Gene Expression (KENGE)-tet system provides a strategy for achieving this. Here I will present the making history of KENGE-tet system and the expanding of the resource repertoire for the glia assembly research.

3-S-01-3 Self-organization strategy in glial cells underlying diverse activity of glia assembly

Hiroko Bannai

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Astrocytes, the most numerous form of glial cell in the brain, have an ability to respond to intercellular messengers with Ca^{2+} signals allowing them to participate actively in the regulation of local blood flow and of synaptic efficacy. Astrocytic Ca^{2+} signals are diverse : some signals are localized in subcellular compartments, while other signals widely spread throughout the cell and even into neighboring cells. The molecular mechanisms enabling such a diverse Ca^{2+} dynamics in single astrocyte, especially that confines Ca^{2+} signals in subcellular compartments, remain to be elucidated. We report here a unique self-organization strategy that underlies localized Ca^{2+} signals in astrocytes. Quantum-dot single particle tracking analysis revealed a membrane barrier that selectively blocked the movement of mGluR5 between astrocyte soma and their processes. We also showed that this barrier contributed to maintain compartmentalized mGluR5 distribution and the localized Ca^{2+} signal. Our results demonstrate an mGluR5-selective diffusion barrier between processes and soma that compartmentalized mGluR Ca^{2+} signaling in astrocytes and may allow control of synaptic and vascular activity in specific subcellular domains.

3-S-01-4 In vivo calcium imaging analysis using a transgenic mice expressing an ultrasensitive calcium sensor

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Astrocytes show dynamic changes in the intracellular Ca^{2+} level (Ca^{2+} signals) that are thought to regulate various brain functions. In vivo Ca^{2+} imaging with high spatiotemporal resolution may pave the way to uncover enigmatic functions of astrocytes. Here we report such method using transgenic mice expressing an ultrasensitive Ca^{2+} sensor protein, YC-Nano50, in astrocytes. This method allowed us to analyze the Ca^{2+} dynamics in individual astrocytes including their fine processes. We found a previously unidentified pattern of spontaneous Ca^{2+} signals (Ca^{2+} twinkles), which are preferably displayed in the fine processes. Upon sensory stimulation, astrocytes in the somatosensory cortex initially responded with Ca^{2+} signals at the fine processes, and the Ca^{2+} signal subsequently propagated to the cell body. Ca^{2+} twinkles and evoked Ca^{2+} signals were partially and fully dependent on the type 2 IP_3 receptor, respectively. These results suggest that astrocytic fine processes function as a high-sensitivity detector of neuronal activities, and indicate involvement of intracellular Ca^{2+} stores in the astrocytic activities. Thus, our present method provides a useful tool to uncover the astrocytic functions.

3-S-02-1 Introduction of analytical methods for data of nonclinical pharmacological studies

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Nonclinical pharmacological studies are conducted for various purposes and, therefore those data have many different characteristics. Furthermore, many analytical methods are applicable to those data and sometimes it would be difficult to choose an appropriate method. In this presentation, I'd like to show several analytical methods using dummy data sets which are based on the studies actually conducted in our laboratory and then, make comparisons with and consideration of the results focusing on which method is 'good' (that is, fit with each data). I hope this presentation would be of some help to pharmacologists carrying out their experiments efficiently.

3-S-02-2 The answers to various questions of when to apply the Williams' multiple comparison test for Pharmacology test

Nobuhiro Nakanishi

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Williams' test is the multiple comparison method that has the advantage of higher power than other multiple comparison methods such as Dunnett's test. However, adoption of the Williams' test in the actual use of pharmacological test is not appreciated enough. One of the reason is that there are the following questions to Williams' test.

1. Interpretation when the dose-response of the observation result is not monotonic
2. Imbalance of each group sample size due to accidental removal
3. Sample size estimation method

I report the knowledge obtained in solving the above questions in order to promote the Williams' test at pharmaceutical company.

3-S-02-3 Parallelism as its premise parallel line assay for efficacy comparison of pharmacological effect

Jun Harada, Michiharu Yoshiike

Dept. Data Science. Div. DAIICHI SANKYO CO., LTD.

Previously, the approach of the parallel line test for potency ratio, have been variousy studied. This situation arises when comparing drug potencies, when studying the action of antagonists, and in numerous other applications in pharmacology and other branches of science.

Today, we will introduce a method that has been taken up in the U.S. Pharmacopeia (USP) bioassay guidance (e.g. Chapter 1032-). In addition, we will introduce a sample case.

3-S-02-4 In-house statistical training for scientists in drug discovery

Chikara Honda

Operations Management, Research Headquaters, Ono Pharmaceutical CO., LTD.

Statistical education should be needed for pursuing good research with rational study designs and appropriate analyses. In the presentation, I'm going to talk examples of in-house practical statistical training for nonclinical pharmacologists and the other scientists. I want to show the knowledge of statistical analysis contribute to progress of drug discovery research.

3-S-03-1 Functional analysis of changes in the mesolimbic dopaminergic system under drug addiction

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Long-lasting usage of abused drugs is considered to induce the functional change in the mesolimbic dopaminergic system projecting to the nucleus accumbence and prefrontal cortex from the ventral tegmental area. We have demonstrated that chronic treatment with abused drugs induces amenability to excitation of the mesolimbic dopamine system associated with an increase in activity of Leucine-rich repeat kinase 2 (LRRK2), which is known to multiply modulate lysosomal positioning and autophagy, synaptic vesicle endocytosis, synaptogenesis, cytoskeleton and neurite outgrowth, protein synthesis, golgi sorting, and retromer function in the ventral tegmental area. Additionally, it up-regulates the down-stream of LRRK2, such as FOXO1, α-synuclein and 4E-BP, which are well-known as regulators of energy metabolism, neurodegeneration and protein synsesis, respectively. Thus, chronic treatment with abused drug may induce the functional change in the mesolimbic dopaminergic system through the modulation of the LRRK2-dependent system. In this symposium, we will discuss the multi-modification of the mesolimbic dopaminergic system by chronic treatment with abused drugs.

3-S-03-2 Mechanism of striatal circuitry underlying behavioral sensitization

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Behavioral sensitization, enhanced motility by repetitive psychostimulant administration, is a model of drug addiction. The nucleus accumbens (Nac) receives dopaminergic afferents from the ventral midbrain and is divided into the shell and core subregions. Each of these contains two subtypes of medium spiny neurons (MSNs), which express either dopamine D1 (D1R) or D2 (D2R) receptor. However, functional diversity between the two subtypes in psychostimulant response has not been fully understood. We carried out selective elimination of each subtype in the Nac shell in mice using immunotoxin-mediated cell targeting and examined the behavioral sensitization evoked by repeated administration of methamphetamine. The D1R cell targeting exhibited delayed induction of sensitized locomotion, whereas the D2R cell ablation showed a mildly enhanced rate of induction of sensitization. In vivo microdialysis indicated a marked blockade of drug-induced dopamine release in the Nac of D1R cell-ablated mice, revealing that the observed delay in behavioral sensitization involves an impairment in accumbal dopamine release. Our results reveal distinct roles of D1R- and D2R-containing accumbal shell MSNs in the development of behavioral sensitization to psychostimulants.

3-S-03-3 Dopaminergic network mechanism in cognitive learning and drug addiction

Takatoshi Hikida

MIC, Kyoto Univ. Grad. Sch. Med.

The basal ganglia are key neural substrates that control not only motor balance but also cognitive learning and decision-making. Dysfunction of the basal ganglia leads to neurodegenerative diseases (e.g. Parkinson's disease and Huntington's disease) and psychiatric disorders (e.g. drug addiction, schizophrenia, and depression). In the basal ganglia circuit, there are two important pathways; the direct and indirect striatal pathways. We have developed a reversible neurotransmission blocking technique, in which transmission of each pathway is selectively blocked by specific expression of transmission-blocking tetanus toxin, and revealed that the activation of D1 receptors in the direct pathway is critical for reward learning/drug addiction, and that the inactivation of D2 receptors is critical for aversive learning/learning flexibility. We also showed that postsynaptic D2L receptors are necessary for cognitive learning and learning flexibility. We propose a new circuit mechanism that the dopaminergic input from ventral tegmental area can switch the direct and indirect pathway in the nucleus accumbens. These basal ganglia circuit mechanisms will give us insights into the pathophysiology of mental diseases.

3-S-03-4 Imaging of dopamine metabolism by mass spectrometry

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Rationale : Highly sensitive neurotransmitter imaging is an important technique for neurodegenerative disease research. So far, positron emission tomography is used for this purpose ; however, it only provides information on specific receptor/ligand interaction. Objective : To develop and validate matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) as a sensitive technique for in situ imaging of neurotransmitters in awaked mouse brain. Methods and Results : Awake mouse brains were fixed in vivo by focused microwave irradiation (FMW). Mapping of amino acid neurotransmitters, glutamate, GABA and glycine were achieve by IMS coupled with recently our developed on-tissue derivatization method (Toue and Sugiura et al., 2014). Furthermore, this imaging technique was proven to be sensitive enough to identify spatial distribution of trace monoamines (e.g., dopamine) on same brain section.Conclusions : Our results highlight the utility and high-sensitivity of MALDI-IMS combined with FMW for visualizing neurotransmitters just as they were in vivo in awaked mouse brains.

3-S-04-1 Current issues in the development of disease-modifying drugs for Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of dementia. AD is neuropathologically characterized by the deposition of amyloid-β (Aβ) and tau proteins. Excessive accumulation of Aβ and hyperphosphorylation of tau play a vital role in the pathogenesis of AD. Aβ has been believed as the most promising target of anti-dementia drugs. Many Aβ-targeted therapies such as β- and γ-secretase inhibitors and Aβ immunotherapy have been developed and tested on humans. However, none of these anti-Aβ drugs has yet to reach success so far. Phosphorylation of tau protein decreases its ability to bind to microtubules, leading to neuronal death. The inhibition of abnormal tau phosphorylation and aggregation appears to be another promising strategy. To facilitate the development of these drugs, it is important to measure the pathologic time course of protein accumulation in the human brain. Recent progress in molecular imaging allows us to visualize both Aβ and tau accumulation in the human brain using positron emission tomography. In vivo Aβ and tau imaging would be useful in monitoring treatment outcomes and for selecting patients for anti-dementia drugs. In this symposium, strategies for successful development of anti-dementia drugs will be discussed.

3-S-04-2 Neurochemical aspects of obstructive sleep apnea syndrome— Medullary serotonergic system—

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The treatment of continuous positive airway pressure (CPAP) is used for moderate to severe obstructive sleep apnea syndrome (OSAS). Although drug therapies can be valuable in mild to moderate OSAS, neurochemical pathophysiology is not clear. Brain serotonergic neurons are CO_2/pH sensors and can be a target of drug therapies in OSAS. The role of medullary serotonin (5-HT) in chemoreceptive airway-ventilatory responses was investigated in the present study using microdialysis and a double-chamber plethysmograph in mice. Mice with a perfusion of a 5-HT2 antagonist through a probe in the dorsomedial medulla oblongata, a part of the respiratory network, inhaled hypercapnic (5, 7, and 9%CO_2 in O_2), hypoxic (7%O_2 in N_2), or hypercapnic hypoxic gases (7%O_2 +5%CO_2 in N_2). Early onsets of hypercapnic or hypoxic airway-ventilatory responses, but not hypercapnic hypoxic responses, were delayed by a perfusion of the 5-HT2 antagonist. These results suggest that chemoreceptive input of CO_2 is compensatory to medullary 5-HT activities in chemoreceptive airway-ventilatory responses. A therapeutic target in mild to moderate OSAS can be 5-HT antagonists, not agonists, although further studies, such as 5-HT's role in CO_2-induced arousal, are needed.

3-S-04-3 Therapeutic strategies for intractable digestive diseases

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Various intractable digestive diseases, such as ulcerative colitis (UC), Crohn's disease (CD), eosinophilic gastroenteritis (EGE), are important therapeutic targets, because the total number of the patients is increased year by year. In addition to such diseases, exponential increase in nonalcoholic steatohepatitis (NASH) is also serious issue in clinical practice. Therefore, establishment of therapeutic strategies based on pharmacological mechanisms for such intractable diseases are really required. For the purpose, we have investigated the potential mechanisms and mechanism-based drug therapy for many intractable digestive diseases. We found the several important mechanisms involved in the pathogenesis of NASH, and therefore, we reported the effective therapies for NASH based on the mechanisms. In contrast, completely mechanism-based drug therapies about the UC, CD, and EGE have not been established yet, although the several effective drug therapies are performed in clinical sites. The reasons may be still-unknown mechanisms and lack of optimal disease models about the intractable digestive diseases. Therefore, investigations using optimal animal models may be required to establish the mechanism-based new therapeutic strategies for the intractable digestive diseases.

3-S-04-4 Development of therapeutics strategy target for intracellular signaling molecules responsible for the pathogenesis of allergic rhinitis

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The expression level of an allergic disease-sensitive gene is correlated with the symptoms severity. Thus, suppression of this gene expression should be good therapeutics. We demonstrated that histamine H_1 receptor (H1R) gene is an allergic rhinitis (AR)-sensitive gene. We also unveiled the signaling pathway responsible for the up-regulation of H1R gene expression. (−)-Maackiain was isolated as an anti-allergic compound that suppresses H1R gene up-regulation, and PKCδ and Hsp90 were identified as novel intracellular target molecules for AR through the molecular pharmacological studies to clarify the mechanism of action of (−)-maackiain. (−)-Maackiain and Hsp90 inhibitors suppressed up-regulation of H1R gene expression and alleviated nasal symptoms in allergy model rats, suggesting the importance of H1R signaling for pathogenesis of AR. However, the fact that decrease in the H1R gene expression to the basal level could not completely resolve nasal symptoms led us to the discovery of the 2nd signaling pathway (Signaling-X) responsible for the pathogenesis of AR. Combination therapy suppressing both H 1R signaling and Signaling-X markedly alleviated nasal symptoms in allergy model rats.

3-S-05-1 Alpha1 adrenoceptor antagonist silodosin suppresses prostatic hyperplasia through an increase in prostatic blood flow

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Chronic ischemia of the prostate could be associated with lower urinary tract symptoms (LUTS). Alpha(α)1-adrenoceptor blockers are widely known that relax prostate smooth muscle and decrease urethral resistance, thereby alleviating benign prostatic hyperplasia related LUTS. We investigated that whether α1-adrenoceptor blocker silodosin could improve blood flow and hyperplasia in the ventral prostate of the spontaneously hypertensive rat (SHR). Twelve-week-old male SHRs were administered with silodosin (100 μg/kg/day, p.o.) or vehicle once daily for 6 weeks. Wistar Kyoto (WKY) rats were used as normotensive controls. The SHRs showed significant increase in the blood pressure, prostate weight, and tissue levels of oxidative stress (MDA), inflammatory cytokines (IL-6, CXCL1/CINC1 and TNF-α), growth factors (TGF-β1 and bFGF), and morphological abnormalities in the ventral prostate compared to WKY rats. On the other hand, the prostatic blood flow was decreased in the SHRs. Treatment with silodosin significantly ameliorated the changes of above parameters in SHRs excluding the blood pressure and prostate weight. Silodosin can suppress the progression of prostatic hyperplasia through an increase of prostatic blood flow.

3-S-05-2 The effective therapy of dutasteride based on new evidence

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Recently, the number of drugs used for treating benign prostatic hyperplasia (BPH) has increased. At present, the combination therapy using an alpha-1 blocker and 5-alpha-reductase inhibitor (5ARI) is recommended for patients with a large prostate. However, many questions remain unsolved. For example, how long the combination therapy should be continued?Whether therapeutic effects decrease or not if the combination therapy is changed to single-drug therapy?
In this symposium, we would like to investigate the effective therapy of dutasteride on BPH patients, based on new evidences of dutasteride.

3-S-05-3 Effects of the phosphodiesterase 5 inhibitor Tadalafil on bladder blood flow and bladder function in rat models

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In men with benign prostatic hyperplasia (BPH), prostate growth leads to partial bladder outlet obstruction (BOO) and makes urination difficult. Patients with BOO secondary to BPH experience ischemia-reperfusion episodes in the bladder during every micturition cycle, with increasing severity of symptoms. There has therefore been a focus on the impairment of bladder blood flow (BBF) as one of the putative mechanisms of obstructive bladder dysfunction. In 2014, the phosphodiesterase 5 inhibitor Tadalafil was approved in Japan for the treatment of lower urinary tract symptoms in patients with BPH. Here, we introduce our research on the effects of Tadalafil on BBF and bladder function in three rat models of ischemia/reperfusion : bladder overdistension/emptying, clamping/release of the abdominal aorta, and BOO (Eur J Pharmacol 2015 754 : 92 ; Neurourol Urodyn 2015, in press). In all rat models, Tadalafil improved BBF, and in BOO rats, the increase in the number of micturitions and the decrease in the average micturition volume were significantly suppressed by Tadalafil. Taken together, these findings suggest that improvement of BBF by Tadalafil may contribute to Tadalafil's amelioration of bladder function.

3-S-05-4 Combination of α1-blockers and anticholinergic agents for prostatic hyperplasia and overactive bladder: Effects and problems

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In prostatic hyperplasia, obstruction of the lower urinary tract causes voiding symptoms. In addition, bladder ischemia associated with this obstruction leads to storage symptoms in 50–75% of cases. Initial drug therapy for prostatic hyperplasia employs α1-blockers, regardless of the presence of storage symptoms. Monotherapy with α1-blockers reduces detrusor overactivity and is thus effective for overactive bladder symptoms; however, this improvement is observed in only 65% of cases. In cases in which α1-blocker monotherapy is ineffective, anticholinergic agents are recommended, yielding an improvement in 73% of cases. However, anticholinergic agents present a risk of adverse events, and therefore should be administered carefully. Drug therapy for prostatic hyperplasia, a disease among the elderly, requires consideration of individual differences in pharmacokinetics, drug interactions, and tolerability. In particular, anticholinergic agents easily cause side effects such as dry mouth and constipation. Furthermore, although rarely, anticholinergic agents can also impair cognitive function. In addition, drug combination therapy involves an increased dose of drugs, putting the patient at risk of polypharmacy.

3-S-06-1 Mechanisms of neurogenesis and gliogenesis to understand pathogenesis of brain diseases

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Generation of neurons starts from the early embryonic stage and continues throughout life in certain brain regions such as the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG). Gliogenesis, i.e., generation of astrocytes and oligodendrocytes, follows neurogenesis in the perinatal stage. It is of note that proliferation of oligodendrocyte precursor cells (OPCs) takes place in the healthy brain, while massive production of astrocytes occurs typically in the injured brain. Extensive studies have been done to elucidate significance of neurogenesisis on various animal behaviors such as learning and memory, but generation of glial cells should deserve to be paid more attention if we consider great impact of recent works on how human astrocytes improve neural functions in the mouse and on how OPCs contribute to mouse cognitive functions. Impairment in neurogenesis seems to be related with mental disorders such as autism, schizophrenia, Alzheimer disease, etc, and so is gliogenesis independently from or synergistically with neurogenesis. Needless to say, there are many molecules and pathways that govern neurogenesis and gliogenesis.

3-S-06-2 Analysis of mechanisms underlying mental and neurodegenerative disorders using the human disease-specific iPS cell technique

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Dopamine neurons are key regulators of emotional and motor coordination. Great efforts are in place to understand the molecular mechanisms that trigger psychiatric and neurodegenerative disorders, taking advantage of human genetics and animal modeling. It has been recognized that most psychiatric and neurodegenerative diseases are sporadic in etiology, arising from what appear to be the complex interactions of genetic and environmental risk factors. Since neuronal dysfunction and degeneration as a result of the neurodegenerative process probably occurs much earlier than the initial neurological manifestations that characterize disease, it may be difficult to fully model these conditions in rodents. Considering these backgrounds, it is useful to apply iPS cells technology for understanding the initiating-process of neurological diseases. In the recent study, we found the dopaminergic cell vulnerability associated with dynamic changes in epigenetics of differentiated dopaminergic neurons derived from Parkinson disease-specific iPS cells. In this symposium, we will discuss the importance of epigenomic landscape for dopaminergic cell vulnerability under mental and neurodegenerative disorders.

3-S-06-3 Generation of blood-brain barrier (BBB) model derived from iPS cells

Kohei Yamamizu

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The blood-brain barrier (BBB) is a network of vessels of brain endothelial cells (BECs) and are ensheathed by pericytes, neurons, and astrocytes. The BBB has the barrier function by forming tight junction and expressing the various transporters for protection of brain homeostasis. We successfully established the efficient differentiation systems of iPS cells into endothelial cells, pericytes, neurons, and astrocytes. The BECs are generated by co-culturing with these four lineages derived from an iPS cell line. The BECs formed the tight junction and highly expressed BBB specific transporters such as GLUT1, PGP, BCRP, and MRP. About 99% of new drugs in development for brain diseases are hampered because these drugs cannot go through BBB. Our technology of BBB would contribute the drug discovery before clinical tests. Furthermore, the extent of BBB impairment in neurodegenerative diseases and how it affects the bioavailability of therapeutics remains unclear. Our BBB models would investigate the molecular mechanisms of progression the neurodegenerative diseases related BBB by using patient-specific iPS cells. Therefore, this technology of BBB model would have a great potential for drug discovery and future therapy of neurodegenerative diseases.

3-S-06-4 Non-human primate as human disease model

Hirotaka James Okano

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Good animal model will play a key role in a significant breakthrough that will greatly impact studies for neurodegenerative diseases aimed at understanding their pathophysiology. The value of Common marmoset (Callithrix jacchus), a small non-human primate, for human disease modeling is well documented during last 15 years, because of its advantage for translation to genetically close human systems. Marmosets, by having smallest-ranged body size and high reproduction rate among primates, are suitable for transgenic modification. Recently, transgenic marmoset was successfully produced by gene transduction into embryos, which creates greatly advantageous animal models to study human neurodegenerative diseases such as Parkinson's disease. Recently, we generated marmoset Parkinson's disease model and conducted MRI analysis. Voxel-based morphometry and diffusion tensor imaging showed decrease of the density in substantia nigra and of projection of nigrostriatal pathway. Furthermore, we established the auditory brainstem response test for study of hearing loss in marmoset. The clinical and pathologic similarities between marmoset disease models and their human counterpart make the non-human primate models potentially valuable for studies on the pathogenesis and treatment of human diseases.

3-S-07-1 Role of mitochondrial ubiquitin ligase MITOL in mitochondrial dynamics and quality control

Shigeru Yanagi

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We have previously identified mitochondrial ubiquitin ligase, MITOL (also known as March5), which regulates mitochondrial dynamics through the ubiquitination of mitochondrial fission factor Drp1 (EMBO J, 2006). Subsequently, we reported that MITOL ubiquitinated and attenuated cell toxicity of unfolded proteins accumulated in mitochondria such as mutant SOD1 and expanded polyglutamine proteins which cause neurodegenerative disorders (Mol. Biol. Chem. 2009, Mitochondrion 2010), suggesting the involvement of MITOL in mitochondrial quality control and pathogenesis of neurodegenerative diseases. To further understand the role of MITOL in mitochondria, we searched for physiological substrates for MITOL and succeeded to identify microtubule-associated protein 1B-light chain 1 (MAP1B-LC1) and mitofusin2 (Mfn2). Recently, we report that MITOL plays a protective role against nitrosative stress-induced mitochondrial dysfunction mediated by MAP1B-LC1 in neuronal cells (PNAS. 2012), and that MITOL is required for ER-mitochondria interaction via Mfn2 (Mol. Cell 2013). In this meeting, by analyzing MITOL-deficient MEFs and mice, we provide evidence for a critical role of MITOL in mitochondrial quality control through regulation of mitochondrial dynamics.

3-S-07-2 Functional coupling and Ca^{2+} signaling between SR and mitochondria in smooth muscles

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The Ca^{2+} signaling microdomain is recognized as a spatiotemporal platform for functional protein assembly and efficient signal transduction in plasmalemmal and subplasmalemmal regions. In smooth muscle cells, depolarization-induced Ca^{2+} influx caused a local $[Ca^{2+}]_{cyt}$ transient (Ca^{2+} hotspot) mediated by Ca^{2+} release from the SR (CICR) at several discrete sites in subplasmalemma areas. Subsequently, the high $[Ca^{2+}]_{cyt}$ within localized area triggered Ca^{2+} uptake of neighboring mitochondria. TIRF imaging revealed that subsets of mitochondria fragments closely localized with RyRs and VDCCs. These results suggest that Ca^{2+} hotspots promote ATP production by the activation of respiratory cycle and the produced ATP is supplied to the coupling SR. Alternatively, confocal fluorescent imaging showed that mitofusin proteins tethered a part of SR and mitochondria each other. In addition, mitofusins were involved in mitochondrial Ca^{2+} uptake following agonist-induced $[Ca^{2+}]_{cyt}$ increase. In conclusion, discrete mitochondria have efficient functional coupling with colocalized SR fragments and plasma membrane as a local Ca^{2+} microdomain and, thereby, substantially contribute to the $[Ca^{2+}]_{cyt}$ regulation in smooth muscles.

3-S-07-3 The analysis of stress responses in mitochondria

Shiori Sekine, Hidenori Ichijo

Cell Signaling, Grad. Sch. of Pharmaceut. Sci., Univ. of Tokyo

Mitochondria not only serve as cellular energy stores but also function as platforms of various stress responses. In this symposium, we will talk about two mitochondrial proteins, PGAM5 and SARM1, both of which are originally identified as activators of ASK1, a MAP3K in the stress responsive MAPK cascades. The first main character of this talk, SARM 1, is a member of TIR domain-containing adaptor molecules. Recently, we found that SARM1 acquires a capacity to activate ASK1 when SARM1 is localized in mitochondria. Our results provide one example that mitochondria can serve as a location for the activation of specific kinase in order to trigger the subsequent proper stress responses. PGAM5, the second main character, is a member of the PGAM family, an evolutionarily conserved enzyme family. Although the amino acid sequence of the catalytic core of other PGAM family members are conserved in PGAM5, we previously reported that PGAM5 lacks mutase activity and instead acts as a Ser/Thr-specific protein phosphatase. Recently, we generated whole-body knockout mice of PGAM5 and found that PGAM5 deficiency confers resistance against several metabolic perturbations. In the second part, we will introduce the novel link between mitochondria-resident protein and whole-body metabolism.

3-S-07-4 Eco-pharma of approved drugs focused on inhibition of mitochondrial fission

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Mitochondrial abnormal metabolism is attracted attention as a major cause of several diseases. We noticed that mitochondrial division was induced prior to cardiomyocyte senescence in the peri-infarct region of myocardium after myocardial infarction in mice. We also found that hypoxic signals were activated in this region, and confirmed that mitochondrial fission was actually induced by 1% hypoxia in rat neonatal cardiomyocytes. Although hypoxic stress induced neither cardiomyocyte cell death nor senescence, reoxygenation of cardiomyocytes significantly induced senescence. Mitochondrial fission was induced by the activation of dynamin-related GTP-binding protein (Drp) 1, and reoxygenated cardiomyocytes showed intracellular ATP accumulation. We performed a drug screening, and found that an ion channel blocker (ICB-1) completely suppressed cardiomyocyte cell senescence induced by hypoxia/reoxygenation. ICB-1 suppressed Drp1 activation and significantly improved heart failure and diabetes in mice. These results strongly suggest that Drp1 activation triggers induction of muscle senescence, and that a novel pharmacological action by ICB-1 has a potency to be additionally applied to mitochondrial excess fission-related intractable diseases.

3-S-08-1 Significance of endogenous and exogenous NO production systems in the pathogenesis of cardiovascular and metabolic diseases

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Nitric oxide (NO) is synthesized by three different NO synthases (nNOS, iNOS, and eNOS). We previously generated mice in which all NOS genes are completely disrupted. The triple NOSs$^{-/-}$ mice manifested metabolic syndrome, myocardial infarction, and diastolic heart failure. Long-term high-cholesterol diet markedly increased plasma LDL cholesterol levels in the triple NOSs$^{-/-}$ mice, and these levels were similar to those in apoE$^{-/-}$ mice. It has been shown that NO is produced from its inert metabolites, nitrite (NO_2^-) and nitrate (NO_3^-). We examined the effect of low nitrite/nitrate diet on metabolic phenotypes in wild-type mice. The long-term low nitrite/nitrate diet resulted in visceral obesity, hypercholesterolemia, impaired glucose tolerance, and insulin resistance in the mice. These results suggest that long-term dietary nitrite/nitrate deficiency causes metabolic syndrome in mice, identifying a specific dietary ingredient that gives rise to metabolic syndrome even in the absence of excess calorie intake. Our findings demonstrate that not only the endogenous NO production system, but the exogenous NO production system also plays a role in the pathogenesis of cardiovascular and metabolic disorders.

3-S-08-2 The Development of Drugs for Natriuretic Peptide System in Cardiovascular Diseases—Bench to Bedside

Tomoko Ichiki, John Burnett

Division of Cardiovascular Diseases, Mayo Clinic

Heart failure (HF) and hypertension (HT) are major health burdens worldwide characterized with impaired neurohumoral systems including reduced activity of natriuretic peptides (NP) with decreased cGMP activation in association with renin-angiotensin-aldosterone-system activation. Stimulation of the NPs receptors GC-A or GC-B has been considered an optimal therapeutic target. Short-term use of NP replacement therapy for acute HF has improved symptoms, while controversy remains regarding improvement of outcomes. LCZ676 (ARNi), which can enhance the endogenous NP system, is approved in the US for chronic stable HF. LCZ676 significantly improved prognosis; however, ARNi may not be efficacious in patients with endogenous NP deficiencies. Thus, endogenous NP deficiency states require direct NP receptor agonists. Our group has been developing chronic NP therapy with subcutaneous (SQ) administration of novel NPs for HF and HT. Because of the challenge of oral peptide delivery, NP therapy requires the half-life and appropriate absorption from subcutaneous area. I will review our experience from bench to bedside the development of CD-NP, a novel dual GC-A/GC-B agonist for HF and MANP, an innovative GC-A agonist for resistant HT.

3-S-08-3 Sigma-1 receptor mediates depressive behaviors induced by cardiovascular diseases

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Cardiovascular diseases are risk factors to cause the depression in human. Since cardiac hypertrophy and heart failure induced by transverse aortic constriction (TAC) was closely associated with the marked reduction of Sigma-1 receptor (Sig-1R) in the brain, we here hypothesized that the impairment of Sig-1R in brain triggers the depressive behaviors in cardiovascular disease patients. Oral administration of a specific Sig-1R agonist SA4503 (0.3-1.0mg/kg) significantly improved TAC-induced depressive behaviors with restoration of Sig-1R expression in both hippocampal CA1 and DG regions. Indeed, the plasma corticosterone levels were significantly elevated in 6 weeks after TAC concomitant with depression-like behavior expression. The chronic corticosterone administration for 3 weeks caused depressive behaviors with the reduction of Sig-1R expression in the hippocampus. Consistent with the improvement of depression behaviors, heart failure was also ameliorated by SA4503 (0.3-1.0mg/kg) administration. Taken together, Sig-1R stimulation with SA 4503 is attractive therapy to improve not only depressive behaviors but also heart failure following cardiovascular diseases.This work is supported by Grants-in-Aid for Scientific Research (Kakenhi 24659024).

3-S-08-4 G protein-coupled receptor-mediated responses on myocardial infarction

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Myocardial infarction occurs by thrombotic occlusion of coronary artery and results in death of cardiac myocytes. During myocardial infarction, it has been known that extracellular pH decreases, that is the concentration of proton increases. Proton is ion but can activate several signaling molecules such as ion channels and G protein-coupled receptors. There are reports that ion channels are activated and play an important role in myocardial infarction-induced cardiac injury. However, the roles of G protein-coupled receptors in myocardial infarction have not been examined so far. There are four members of the receptors that are regulated by proton. These are named as proton-sensing G protein-coupled receptors. Each receptor has unique cellular distribution and activates distinct signaling pathways to cause detrimental or protective changes in the cells. In this presentation, I would like to introduce the roles of the proton-sensing receptors in myocardial infarction, and suggest proton-sensing receptors are a promising target for treatment of myocardial infarction.

3-S-09-1 Prediction method of cardiotoxicity triggered by kinase inhibition

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Although tyrosine kinase inhibitors (TKIs), belonging to molecularly targeted drugs, have been designed to inhibit particular kinases, adverse drug reactions (ADRs) by TKIs are difficult to predict since they are considered to be associated with off-target inhibition. Therefore, for predicting ADRs, clarification of comprehensive inhibition profile of kinases by TKIs and identification of kinases highly relevant to ADRs are important. The former is evaluable by comprehensive affinity measurement method reported previously. To address the latter, systems biological analyses are adopted here. In this study, cardiotoxicity has been focused due to its clinical severity. Since some TKIs are reported to cause cardiac cell apoptosis leading to cardiotoxicity, the information of intracellular signaling pathways from various kinases to apoptosis in cardiac cells were collected from knowledge bases and was integrated into a large single map. With applying dynamic simulation based analysis, risks of various kinases on apoptosis are evaluated. The calculated risks show good correlation with viability of primary cultured cardiomyocytes under knockdown of several kinases, suggesting that our method could be helpful in identification of high risk kinases for cardiotoxicity.

3-S-09-2 Cardiactoxicity Prediction of drug using Three-dimensional Heart Simulator

Jun-ichi Okada[1,2], Takashi Yoshinaga[3], Junko Kurokawa[4], Takumi Washio[1,2], Tetsushi Furukawa[4], Kohei Sawada[3], Seiryo Sugiura[1,2], Toshiaki Hisada[2]

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We will show a novel cardiotoxicity testing system combining in vitro ion current measurements using the patch clamp technique and a three-dimensional, multi-scale, multi-physics heart simulator (UT-Heart). This simulator implements the excitation-propagation and excitation-contraction coupling process, based on a finite element method with parallel computation. Because the heart model, torso model, and conduction system model are coupled by a multi-scale algorithm we can analyse the changes in the electrocardiogram caused by modification of ion channels by drug effects. To evaluate the pro-arrhythmic risk of drugs, the guideline for drug safety testing (ICH-S7B) recommends measurement of IKr through the hERG channel, as well as an in vivo QT assay and examination of the QT interval for drug development. Our heart simulator more accurately predicted the pro-arrhythmic risk than the QT interval and the HERG test. The inhibitory effects of drugs are analysed for the six or seven ion channels, and the effects of drugs under various plasma concentrations are reproduced by changing parameter values of 22 million cell models implemented in the heart model.

3-S-09-3 In silico study for meeting the needs of safety pharmacological evaluation: Models of the rapid pharmacological evaluation and the induced pluripotent stem cell-derived myocardium

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To improve the cost-efficiency of drug development and to decrease the number of animal testing, both human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) and *in silico* approach (computer simulation) are attempted to be used for the evaluation of safety pharmacology in the process of drug development. However, whether such novel approaches properly evaluate the clinical pharmacological effects is still unclear. Indeed, it has been known that the electrophysiological properties of hiPSC-CM differ in some respects from those of the original human heart. Moreover, there is a limited number of specialists in the field of *in silico* pharmacology (or arrhythmology), and thus in some cases *in silico* models are not handled properly. The purposes of this talk are to give an overview of some of *in silico* studies applicable to the cardiac safety pharmacology testing (including the analysis of a gap between the hiPSC-CM and the real human heart) and to comment on the possible future direction of the *in silico* safety pharmacology.

3-S-09-4 Significance of comprehensive and quantitative proteomics of membrane transport proteins in drug discovery

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More than 60% of approved drug-targets are membrane proteins. Among membrane proteins, membrane transport proteins, such as transporters or channels, play critical roles for living cells. While number of membrane transport protein as a target of the existing drugs is limited, most of the drugs targeting transport proteins are quite important. Moreover, it is predicted that more membrane transport proteins would be targets of new drugs. Thus, it is very meaningful to obtain comprehensive expression profiles of the membrane transport proteins in various cells or tissues and quantitate the proteins accurately. However, due to hydrophobic properties of the transport proteins, comprehensive expression analysis of the proteins is difficult and the quantification is performed rarely. Recently, we have developed a proteomic procedure that allows us to analyze transport proteins comprehensively and quantitatively. The comprehensive and quantitative proteomics lead us to the new stage of drug discovery targeting transport proteins as well as that of in silico modeling.

3-S-10-1 Regulation of excitation-contraction coupling by junctional membrane-associated proteins

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Skeletal muscle L-type calcium channels (LTCC) are specifically localized to the junctional membrane (JM) where the sarcolemma are closely apposed to the sarcoplasmic reticulum. Although the targeting of LTCC is critical for efficient excitation-contraction coupling, its molecular mechanism has not been clarified. Junctophilins (JPs) are known to stabilize the JM complex by bridging the plasma membrane and sarcoplasmic reticulum. We explored structural and functional consequences of JP knockdown (KD) in C2C12 and GLT myotubes. JP1 or JP2 KD significantly inhibited the JM-targeting of LTCC whereas JP2 but not JP1 KD significantly decreased the current density of LTCC. Calcium imaging assay showed that JP1 or JP2 KD significantly decreased the number of myotubes exhibiting calcium transient in response to electrical stimulation. Co-immunoprecipitation and GST pull down assay showed that JP-binding domain (JPD) exists and is located in the proximal C-terminus of $Ca_V1.1$. Immunocytochemical analysis revealed that the JM-targeting rate of the JPD-mutated $Ca_V1.1$ was significantly reduced compared with the wild type. These results suggest that interaction of $Ca_V1.1$ with JPs via JPD is important for the proper localization and function of LTCC.

3-S-10-2 Ryanodine receptor/Ca^{2+} release channel-linked diseases in skeletal muscle

Takashi Murayama

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The type 1 ryanodine receptor (RyR1) is a Ca^{2+} release channel in the sarcoplasmic reticulum of skeletal muscle and is mutated in several diseases, including malignant hyperthermia (MH) and central core disease (CCD). Most MH and CCD mutations cause accelerated Ca^{2+} release, resulting in abnormal Ca^{2+} homeostasis in skeletal muscle. However, how specific mutations affect the channel to produce different phenotypes is not well understood. We have recently developed a method to quantitatively evaluate the CICR activity of RyR1 channels using [^{3}H]ryanodine binding and ER luminal Ca^{2+} measurements (Murayama T, Kurebayashi N et al., PLoS ONE, 10 : e0130606, 2015). Using this method, we analyzed RyR1 channels carrying different MH and CCD mutations in the amino-terminal and central regions by expressing them in HEK293 cells. The mutations divergently affected two parameters for CICR, i.e., the gain that determines the attainable maximum activity and the sensitivity to activating Ca^{2+} in a site-specific manner. The CICR activity of the mutants well correlated with the severity of diseases. Our method can also be applied to diagnosis and treatment of the diseases. I will talk about recent progress of our research and approach toward diagnosis and therapy.

3-S-10-3 Mouse models for myopathy associated with nuclear envelopathy

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Mutations in the genes encoding nuclear envelope proteins cause several diseases, so called nuclear envelopathy. Deficiency of emerin, an inner nuclear membrane protein, cause Emery-Dreifuss muscular dystrophy (EDMD), which is clinically characterized by slowly progressive muscular dystrophy, cardiomyopathy with conduction defects, and early joint contractures. Mutations in the LMNA gene, which encodes A-type lamins, cause variable diseases including EDMD, limb girdle muscular dystrophy, partial lipodystrophy, and premature aging. To date, several mouse models for nuclear envelopathy have been developed, but there is no good model mimicking myopathy observed in EDMD. We produced a new mouse model (EH) by crossing Emd knockout (Emd) and Lmna H222P knock-in (H222P) mice.EH mice show prominent degeneration of skeletal muscle is seen together with cardiac fibrosis in EH mice. Surprisingly, cardiac fibrosis in EH mice is milder than H222P mice, which died by prominent dilation of heart with plural effusion.This new animal model is useful to elucidate the different roles of nuclear envelope proteins in both skeletal and cardiac muscles.

3-S-10-4 Molecular mechanism of sarcopenia—Adaptive changes in negative regulators modulating muscle mass

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Sarcopenia, the age-related loss of skeletal muscle mass, is characterized by a deterioration of muscle quantity and quality leading to a gradual slowing of movement, a decline in strength and power, increased risk of fall-related injury, and often, frailty. Since sarcopenia is largely attributed to various molecular mediators affecting fiber size, mitochondrial homeostatis, and apoptosis, the mechanisms responsible for these deleterious changes present numerous therapeutic targets for drug discovery. Muscle loss has been linked with several proteolytic systems, including the ubuiquitin-proteasome, lysosome-autophagy, and TNF-a-NF-kB (nuclear factor-kappaB) systems. Although many factors are considered to regulate age-dependent muscle loss, this gentle atrophy is not affected by factors known to enhance rapid atrophy (denervation, hindlimb suspension etc). In addition, defects in SRF (serum response factor)-dependent signaling have been found in sarcopenic muscle. Intriguingly, more recent studies indicated an apparent functional defect in autophagy- and myostatin-dependent signaling in sarcopenic muscle. In this symposium, we summarize the current understanding of the adaptation of many regulators in sarcopenia.

3-S-11-1 Early weaning increased emotional behavior in mice

Takefumi Kikusui

School of Veterinary Medicine, Azabu University

We examined the developmental effects of early weaning on anxiety and the extinction of fear memory in male C57BL/6 mice. Early weaning led to increased freezing behaviors after fear conditioning via the foot-shock method both during extinction training and in a test of extinction retrieval. In addition, we found that the levels of brain-derived neurotrophic factor (BDNF) protein and mRNA transcripts for BDNF exon III in the medial prefrontal cortex (mPFC) at the time of extinction retrieval were lower in early-weaned mice than normally-weaned mice. Furthermore, BDNF expression was negatively correlated with the duration of freezing behaviors during extinction training. Together, these findings suggest that early weaning impaired fear extinction, and this was correlated with a decrease in BDNF protein levels in the mPFC that was caused by disrupted BDNF protein synthesis from its mRNA transcripts with or without reduced transcription of BDNF Exon III. These data suggest that early weaning of C 57BL/6 mice might provide a reliable animal model for studies of the pathogenesis of post-traumatic stress disorder.

3-S-11-2 Effects of early life stress on the brain

Mayumi Nishi, Takayo Sasagawa, Noriko Horii-Hayashi

Dept. Anatomy & Cell Biol., Nara Med. Univ.

Many studies have shown that daily repeated maternal separation (MS), an animal model of early life stress, can regulate the hypothalamic-pituitary-adrenal axis (HPA axis) and affect subsequent brain function and behavior during entire life including puberty and adulthood. However, the molecular basis of the long-lasting effects of early life stress on brain function has not been fully elucidated. In this symposium, we will present various cases of MS in rodents and illustrate the alterations in HPA axis activity by focusing on corticosterone (CORT). We then show a characterization of the brain regions affected by various patterns of MS, including repeated MS and single time MS at various stages before weaning, by investigating neuronal activity marker, c-Fos. These CORT and c-Fos studies suggest that repeated early life stress may affect neuronal function in region- and temporal-specific manners, indicating a critical period for habituation to early life stress. Next, we introduce how early life stress can impact behavior, namely by inducing depression, anxiety or eating disorders, and alterations in gene expression in adult mice subjected to MS.This study was supported by Grants-in-Aid for Scientific Research from the JSPS.

3-S-11-3 Impact of environmental factors in early life on brain development and emotional behaviors

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Epidemiological researches of neurological and psychiatric disorders have provided the importance of environmental factors, especially during early life, in mental health. Accordingly, there are a growing number of animal studies to imply the involvement of environmental factors in brain and mental development. We have previously demonstrated environmental enrichment (EE), which is considered to enhance motor, sensory and cognitive stimulation (Nat. Rev. Neurosci., 2006), during early developmental period attenuates abnormalities of emotional behaviors in adult pituitary adenylate cyclase-activating polypeptide-knockout (PACAP-KO) mice (Behav. Brain Res., 2010 ; 2014). Furthermore, we have recently revealed that factors other than mortar stimulation are important in the improvement of behavioral abnormalities by EE in PACAP-KO mice via a BDNF-independent increase in dendritic spine density in the hippocampal CA1. The present review summarizes the roles of environmental factors in early life on brain development and emotional behaviors, focused on our findings on regulation of the expression of behavioral abnormalities by early life EE in mouse models of psychiatric disorders.

3-S-11-4 Early life stress produces stress vulnerability and affects chronic pain

Takashi Nishinaka, Kazuo Nakamoto, Shogo Tokuyama

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Clinical studies have demonstrated that adversity in early life increases the risk of developing a chronic pain in adulthood, suggesting that early life stress increases the vulnerability to development of chronic pain. Neuropathic pain leads to impairment of the neuronal function in multiple brain regions which contribute to the maintenance of pain and development of emotional dysfunction. Therefore, early life stress-induced abnormalities of neuronal function in brain may negatively affect the pain modulation system during neuropathic pain condition. In the present study, we investigated the behavioral and molecular effect of early life stress on the model mice of neuropathic pain. In our model, early life stress exacerbated the hyperalgesia and emotional dysfunction after neuropathic pain in adult mice. Transient alteration of brain derived neurotrophic factor (BDNF) levels in the brain was observed immediately after early life stress but not nerve injury. Furthermore, early life stress increased p-ERK, a neuronal activation maker, expression in the multiple brain regions of adult mice. These findings suggest that early life stress negatively affect the neuropathic pain through the abnormalities of neuronal function in brain.

3-S-12-1 Anti-atrial fibrillatory effect of anti-influenza drug oseltamivir

Yuji Nakamura, Takeshi Wada, Hiroko Izumi-Nakaseko, Kentaro Ando, Atsushi Sugiyama

Dept. Pharmacol., Fclt. Med., Toho Univ.

Oseltamivir is a potent and selective neuraminidase enzyme inhibitor. The drug has been recently shown to more selectively delay the intra-atrial conduction than the intra-ventricular conduction in the halothane-anesthetized dogs, which was less selective for pilsicainide. We assessed the anti-atrial fibrillatory effect of oseltamivir by using recently developed persistent atrial fibrillation model of beagle dogs. Class I drugs (3 mg/kg of disopyramide, aprindine, cibenzoline and pilsicainide), I_{Kur} blocker (6 mg/kg of AVE0118), and 3 and 30 mg/kg of oseltamivir were intravenously administered to the model over 10 min (n=6 for each drug). Disopyramide, aprindine, cibenzoline, pilsicainide and AVE 0118 terminated the atrial fibrillation in 1, 0, 2, 2 and 2 out of 6 animals in each group, respectively. The low and high doses of oseltamivir terminate the atrial fibrillation in 2 and 5 out of 6 animals, respectively. It should be noted that disopyramide induced torsade de pointes following remarkable QT-interval prolongation in 1 dog without terminating the atrial fibrillation. Thus, oseltamivir may become a efficacious and safe strategy for treating atrial fibrillation.

3-S-12-2 Supplemental indication approval by an application based on public knowledge for a currently approved drug: history and current status in Japan

Shinichi Kawai

Div. Rheumatol., Dept. Intern. Med., Toho Univ. Sch. Med.

History and current status of the supplemental indication approval by an application based on public knowledge for a currently approved drug will be introduced in this symposium.

3-S-12-3 Drug Repositioning based Drug Development: —From Pharmaceutical Industry's viewpoints—

Masanori Osakabe

Nobelpharma, R&D Division

Drug Repositioning (DR) is a new methodology to apply known drugs and failed investigational products to new indications different from their original indications. Nobelpharma is a new company established in 2003, targeting necessary but neglected drugs and medical devices that are usually indicated for rare diseases with small market sizes. Consequently, Nobelpharma has been applying DR approach to get approvals.In this symposium, I will introduce some of our drugs developed by DR approach and will try to give careful consideration to DR-based approach to promote a better understanding of the obstructive factors against developing new drugs for rare diseases.

3-S-13-1 Toxicity evaluation on a bioartificial liver using ^{13}C-glucose breath test

Tomokazu Matsuura

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The major cause of candidate compounds dropout in drug discovery is toxic effects in the liver caused by hepatic drug metabolism and pharmacokinetics. We are developing the prediction system on human drug metabolism and pharmacokinetics using a bioartificial liver (BAL), which is contracted by high density human liver cells cultured in a 3-D radial-flow bioreactor (RFB). This system is useful in studying drug interactions during induction of human CYP3A4. The CYP3A4 mRNA after rifampisin treatment in the BAL was 100 times higher than in a monolayer culture of a human cell line, FLC-5.We also made a 3-D reconstructed liver organoid using RFB, and evaluated viability of the organoid by ^{13}C-glucose breath test (GBT). The liver organoid was constructed by co-culture with immortalized mouse hepatocytes, sinusoidal cells. Exhaust gas was sampled in gas bags from the closed circuit of RFB system. Firstly we observed transition of $^{13}CO_2$ in exhaust gas along culture time. Glucose metabolism was enhanced by not only hepatocyte growth, but co-culture of non-parenchymal cells. We also examined ischemia/reperfusion injury model in the reconstructed liver organoid. ^{13}C-GBT clearly showed depressed metabolism by ischemic effect to the liver organoid.

3-S-13-2 Evaluation of various pathophysiological functions by the breath test using ^{13}C-labelled compounds in small animals

Orie Kobayashi, Masayuki Uchida

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In the present study, we introduce the methods evaluating various physiological functions by the breath test using ^{13}C-labelled compounds in small animals. This method has a merit to be able to evaluate in course of time without sacrificing animals and in the non-anesthetized and non-restraint state. A desiccator was employed so that the rats could move freely within the chamber, and the expired air could be collected effectively in the breath-sampling bag using pump. POCone was chosen to measure the expired ^{13}C-labelled air simply and effectively (Uchida *et al*. J Pharmacol Sci. 2005). Gastric emptying and gastrocecal transit time were evaluated by using ^{13}C-acetic acid or ^{13}C-octanoic acid and lactose-^{13}C-ureide, respectively. N-Benzoyl-L-tyrosyl-1-^{13}C-L-alanine sodium was used for studying pancreatic exocrine function. The metabolism of ^{13}C-labelled carbohydrate, lipid, amino acid and peptide was also estimable. Pathophysiological functions and drug's effects could be evaluated. With the use of another or newly synthesized ^{13}C-labelled compounds, further physiological function, pathopysiological state or drug's effects would be clarified by using the present method.

3-S-13-3 A rodent model of impaired gastric motility resulting from gastric low-grade inflammation induced by allyl isothiocyanate, a pungent ingredient of wasabi, for drug evaluation: the stable isotope-labeled compound [^{13}C]-acetic acid breath test

Kimihito Tashima[1], Kenjiro Matsumoto[2], Kazuki Hashimoto[1], Noriyuki Oshi[1], Taku Imai[1], Syunji Horie[1]

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We have shown the gastrointestinal pharmacological effects of a wasabi ingredient allyl isothiocyanate (AITC), which induced the low-grade inflammation in rat stomachs. This study is to establish a mouse model of impaired gastric motility induced by AITC, which is reliable produce to evaluate prokinetic agents by detecting the stable isotope [^{13}C] in the breath. Male ddY mice were used after 15 h-fasting. Gastric motility was measured by [^{13}C]-acetic acid breath test in conscious mice. AITC was given p.o. 30 min before the measurement of motility. Prokinetic agents including itopride, mosapride, and neostigmine were given s.c. 40 min before the measurement. AITC impaired gastric motility in dose-dependent manner in mice, yet gastric damage was not observed by AITC. Decreased gastric motility induced by AITC was restored by the pretreatment of itopride, mosapride, and neostigmine. These results demonstrate that impaired gastric motility induced by AITC was reversible by prokinetic agents in conscious mice, which might be a useful animal model for drug evaluation of prokinetic agents.

3-S-13-4 Current status and perspective in clinical application of biological function test uing ^{13}C-breath test

Koji Nakada[1,2], Masahiko Kawamura[1,2], Hideo Konishi[2], Taizo Iwasaki[2], Keishiro Murakami[2], Atsuo Shida[2], Norio Mitsumori[2], Tomokazu Matsuura[3], Nobuyoshi Hanyu[2], Katsuhiko Yanaga[2]

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Basic and clinical medical research using ^{13}C-breath tests have been performed over four decades, however, clinical application of these useful biological function tests are not yet widely prevailing and these tests are still remain under consideration of covered by insurance. ^{13}C-breath test has several advantages including non-invasiveness, simplicity and safety and may contribute to investigate the pathophysiology of various disease and to evaluate the severity of the disease by obtaining new dimensional data about biological function. In current status, ^{13}C-breath test is used to evaluate several biological function such as gatric emptying, fat absorption, exsocrine pancreatic insufficiency, liver metabolism, insulin resistance, small intestinal bacterial overgrowth, colonic mucosal injury in inflammatory bowel disease as clinical research, then, more expansion of the clinical application of ^{13}C-breath test in dairy practice is anticipated. For this purpose, to standardize the method, to fix the standard, to disperse the usefulness of the tests and to be covered by insurance are vital. In the presentation, the current status of several biological function tests using ^{13}C-breath test and perspective of those will be shown.

3-S-14-1 Optogenetic control of central serotonergic neurons affects impulse control and model-based decision making

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It has been speculated that serotonin release in the forebrain is involved in impulse control and model-based decision making. However, there is so far no direct evidence proving this hypothesis. To obtain the direct evidence, we developed transgenic mice expressing channelrhodopsin-2 (ChR2) mutant (C128S) or Archaerhodopsin T (ArchT) only in central serotonergic neurons. A 3-choice serial reaction time task (3-CSRTT) was used to assess impulsive action. A lithium devaluation task was used to assess model-based decision making. In this paradigm, a mouse is first trained to poke its nose to illuminated holes to get a food pellet, and then the food is devalued by pairing it with lithium-induced illness. Optogenetic activation of serotonergic neurons suppressed impulsive action without affecting other cognitive/motor parameters in the 3-CSRTT. Although inconclusive, preliminary results indicated that optogenetic silencing of serotonergic neurons impaired model-based decision making. Thus it is likely that central serotonergic activity has a pivotal role in impulse control and model-based decision making.

3-S-14-2 Neural computation mechanism of prediction and decision making by dorsal raphe serotonin neurons

Katsuhiko Miyazaki, Kayoko Miyazaki, Kenji Doya

Okinawa Institute of Science and Technology

Recently we showed that optogenetic activation of the serotonin neurons in the dorsal raphe nucleus (DRN) enhanced the mice's patience in waiting for reward. Here we test if the probability of future rewards affects promotion of patience. Mice were trained a task with a 75% probabilistic food pellet after 3 s delay. We focused on the waiting time of 25% of omission trials. In half of the trials, DRN serotonin neurons were optogenetically activated during waiting for rewards. In the omission trials, the waiting time with serotonin neuron activation was significantly longer than that without activation. Next, the mice performed a with a 25% probabilistic food reward after waiting for 3 s. In the omission trials, the waiting time with serotonin activation was not significantly different from that without activation. Finally we introduced a 25% reward test with three pellets, in which the expected reward value was equated with that in the 75% reward test. The waiting time in the omission trials with serotonin activation was not significantly different from that without serotonin activation. These results suggest that serotonin's effect on promoting patience depends on a high probability, but not expected value, of future reward.

3-S-14-3 Dynamic encoding of appetitive and aversive information in the primate dorsal raphé nucleus

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Dept. Physiol. Kansai Medical Univ. Sch. Med

There have been inconsistent hypotheses about whether and how the central serotonergic system is involved in positive or negative emotional information processing. The dorsal raphé nucleus (DRN) is the major source of serotonin in the forebrain, and despite neurochemical variability, measurement of single DRN neurons' activity would provide significant understandings of such opposing information processing. We therefore performed single unit recording from the DRN while monkeys were conditioned in a Pavlovian procedure with two distinct contexts : an appetitive context where a reward was available and an aversive one where an airpuff was delivered. We found that the DRN neurons discriminate appetitive and aversive contexts by tonic modulation in activity. Using the eye movement tasks, we also found that the same neurons further kept track of moment-to-moment changing expected reward value in the appetitive, but not in the aversive context. Such temporally distinct DRN neuronal activities may provide the neural basis of information processing required for decision making in different emotional contexts.

3-S-14-4 A role of the dorsal raphe serotonin neurons in the regulation of emotion

Kazuki Nagayasu[1,2], Shuji Kaneko[2]

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Serotonin (5-HT) have been thought to play an important role in regulation of emotional states. Indeed, a variety of therapeutic agents for mood disorder modulate the 5-HT signal transmission. We have previously reported that the activity of the 5-HT neurons in the dorsal raphe nucleus (DRN) can be increased by ketamine and olanzapine, promising therapeutic agents for treatment-resistant depression, as well as conventional antidepressants such as selective serotonin reuptake inhibitors and tricyclic antidepressants. It is, however, still unclear whether the activation of the DRN 5-HT neurons is sufficient for eliciting antidepressant-like effects. Furthermore, identification of responsible 5-HT circuits for a variety of behaviors and disorders has been hampered by wide-ranging projection of 5-HT neurons. To address these issues, we developed lentiviral vectors capable of optogenetic manipulation of 5-HT neurons specifically. In this symposium, we would like to show our recent findings on the role of 5-HT neurons in the regulation of depression and anxiety.

3-S-15-1 Economic evaluation of team care—Pharmacological impact of pharmacist participation in a multidisciplinary team—

Shunya Ikeda

International Univ. Sch. Pharm.

There are many foreign studies which directly or indirectly demonstrated the economic impact of clinical pharmacy services. Viswanathan et al. reviewed 44 studies which assessed the effect of Medication Therapy Management (MTM) interventions among outpatients with chronic illnesses. MTM interventions improved medication appropriateness and adherence, and reduced medication dosing. They also reduced health plan expenditures on medication costs and hospitalization costs.
Based on the evidence from the foreign studies, I will present the pharmacological impact of the pharmacist participation in a multidisciplinary team and the possibility of its economic benefit in the Japanese clinical setting.

3-S-15-2 From the standpoint of pharmacists working for medical insurance pharmacies focusing on improvement of adherence

Yuko Doi

Ain Holdings Inc.

Since Japan has become super-aged society, pharmacists need to consider drug therapy that is appropriate for elderly people. Reconsideration of conventional drug therapy is required to deal with problems specific to elderly people, such as bone fractures, swallowing disorders and dementia, all of which cause decreases in the level of QOL. Pharmacists must give attention also to various aspects of living environments of elderly patients.In this symposium, I will present several cases of patients, who are subjected to home medical care, and discuss about effectiveness and problems of home medical care provided by a regional medical network from a pharmacological point of view. As a result of examining the cases in detail, I reached a conclusion that one solution to the problems might be obtained by sharing information about patients and their living conditions among pharmacists and other medical professionals working in the same regional medical network. Under these conditions, pharmacists should propose drug therapies that are most suitable for elderly patients.

3-S-15-3 New pharmacy practice based on interprofessional work is developing in Kobe University Hospital

Midori Hirai

Dept. Hosp. Pharm., Kobe Univ. Sch. Med.

Today, to achieve optimal patient outcomes, the focus in healthcare has been shifted towards patient-centeredness approaches to achieve optimal patient outcomes. Pharmacists are at the forefront of patient pharmaceutical care which improves pharmacological and therapeutic outcomes with by minimizing adverse events of induced by drug use. In our hospital, pharmacists are working as active members of many care teams developing interprofessional work. Pharmacists should promote take leadership in pharmacotherapy for patient outcome and safety as well as reduction of incidence of adverse drug reactions. I will introduce the pharmacists' activity activities in the interprofessional education for undergraduate students, in outpatient clinics as a member of interdisciplinary teams, and in medication assessment using STOPP/START criteria for avoiding inappropriate prescribing.

3-S-15-4 Characteristics of effective interprofessional medical teams and usefulness of team training to promote effective health care

Tomohiro Taguchi

Dept. Family Medicine, Mie Univ. Sch. Med.

Recently, the importance of interprofessional medical team(s) (IMT) has been recognized widely due to such factors as increasing complexity and specialization of medical care, the increasing incidence of chronic disease, shortage of global workforce and initiatives for safe working hours, etc.
According to many studies, it has been proven that effective IMTs can provide better quality of medical care at the organization level such as reduced hospitalization time and costs, the patient level such as improved health outcomes and reduced medical errors, the team level as a whole such as improved coordination of care and the individual team member level such as enhanced job satisfaction.
Effective IMTs are never formed by accident, therefore we need first to understand characteristics of successful teams and to know how they function and maintain their effectiveness, and, secondly, to carry out training programs essential to promote effective health care.
Based on the evidence obtained from various medical studies and some cases under Japanese clinical settings, I demonstrate characteristics of effective IMTs and usefulness of team training to make excellent IMTs.

3-S-16-1 Disruption of gap junction as the therapeutic target in sensorineural hearing impairment

Taro Yamaguchi, Masanori Yoneyama, Kiyokazu Ogita

Dept. pharmacol., Setsunan Univ.

Most of sensorineural hearing impairment, which causes the inner ear dysfunction, is irreversible and has become a social problem because there is no treatment of sensorineural hearing impairment. Under physiological conditions, maintenance of the potassium ion concentration in the endolymph by gap junction in the cochlear lateral wall is important for hearing. We found using in vivo and in vitro experiments that intense noise exposure produced disruption of gap junction through oxidative stress-induced connexin degradation prior to hair cell damage. Therefore, the collapse of ion balance in the inner ear through degradation of connexin is one of the causes of hair cell damages. In addition, calpain inhibitors prevented noise-induced hearing loss and disruption of gap junction induced by connexin degradation. Taken together, the disruption of gap junction by oxidative stress is involved in one of the pathogenesis of sensorineural hearing impairment and could be a new target of pharmacotherapy for sensorineural hearing impairment.

3-S-16-2 Generation of a new mouse model for deafness by optogenetic approach

Hiroshi Hibino[1], Mitsuo Sato[1], Fumiaki Nin[1], Kenji Tanaka[2]

[1] Dept. Mol. Physiol., Niigata Univ. Sch. Med., [2] Dept. Neuropsy., Sch. Med., Keio Univ.

More than 10% of world population currently suffer from hearing loss, which is primarily caused by disorders in the inner ear in both congenital and acquired conditions. In the latter, some cases that show reversibility at the early stage become irreversible and finally result in refractory hearing loss. To develop new drugs and therapeutic strategies for the disease, creation of model animals that mimic reversible sensorineural hearing loss in human is crucial. For such purpose, in this study we have characterized the transgenic mice that expressed channelrhodopsin-2 in the stria vascularis. This epithelial tissue maintains a highly positive potential of +80 mV in the cochlear endolymph, a K^+-enriched extracellular solution essential for audition. Hearing threshold of the mice was increased by ~20 dB when their cochleae were exposed to blue light. Electrophysiological experiments showed that the illumination sharply reduced the endolymphatic potential by 25 mV in a few seconds. Upon cessation of the light exposure, the EP was completely recovered within 5 minutes. The extent of the EP reduction depended on duration and intensity of the illumination. The mice may serve useful tools to study reversible or transient hearing disorders.

3-S-16-3 Protection and regeneration of cochlear hair cells using IGF1 and its downstream molecule

Norio Yamamoto

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We have demonstrated that insulin-like growth factor1 (IGF 1) protected cochlear hair cells (HCs) of neonatal mice against aminoglycoside (AG) through the inhibition of apoptosis and the induction of cell cycle promotion. Moreover, we have confirmed its effectiveness to idiopathic sudden sensorineural hearing less in the clinical trial. As effectors of IGF1 signaling during hair cell protection against AG, we have identified two candidate genes, *Gap43* and *Ntn1* (netrin-1) using microarray and quantitative RT-PCR. The netrins constitute a conserved family of secreted proteins that are involved in axon guidance and cell migration. Recent studies reported other roles of NTN1 including tissue morphogenesis or an anti-apoptotic survival effect. To examine the effect of NTN1 on cochlear hair cells, we utilized cochlear explant culture established from P2 mice and found that NTN1 protected HCs from AG damage through inhibition of apoptosis. This effect of NTN1 was mediated by UNC5B, one of the six canonical NTN1 receptors. Moreover, we confirmed that the effect of IGF1 was attenuated by blocking NTN1 or UNC5B. These results suggest that NTN1 is one of the effectors of IGF1 in the cochlea.

3-S-16-4 Analysis of hearing loss causing from failed maintenance of auditory hair's integrity

Takehiko Ueyama, Naoaki Saito

Lab. of Molecular Pharmacol., Biosignal Research Ctr., Kobe Univ

Cochlear hair cells (CHCs) have specialized structures, called stereocilia (auditory hairs) which are composed of hundreds of actin filaments, on the apical surface. Stereocilia are graded in length and organized into a characteristic staircase patterns. Each stereocilia bundle displays a tightly regulated size and shape : a narrow base and almost cylindal upper part. High sensitivity of CHCs depends on the coordinated movement of stereocilia ; thus, the length and shape of stereocilia are determined precisely. Moreover, as CHCs are usually not replaced, turnover of stereociliary components is a lifetime requirement.Cdc42 is a member of Rho-family small GTPases. We reported HC-specific conditional KO mice whose CHCs normally developed but progressively degenerated after maturation, resulting in progressive hearing loss. Cdc42 is localized and active at stereocilia for their maintenance, but not development. We further developed our study based on the hypothesis that other Rho-family small GTPases may play important roles in development of stereocilia. We also investigated downstream molecules of Cdc42 signaling. In this symposium, we will show and disscuss our current results about development/maintenance of stereocilia.

3-S-17-1 Central regulation of hepatic metabolism by leptin

Licht Miyamoto

Dept. Med. Pharmacol., Inst. of Biomed. Sci. Tokushima Univ.

Leptin is an adipocytokine which enhances glucose and lipid metabolism as well as suppresses appetite. Especially, leptin administration demonstrates the striking beneficial effects against metabolic disturbances in lipodystrophy, which is a disease due to lack of adipose tissue, characterized by multiple severe metabolic abnormalities including diabetes and fatty liver. Thus leptin is clinically applied to treat the metabolic disorders in lipodystrophy. We have recently found that leptin administration activates hepatic AMPK, which is a key molecule controlling energy metabolism, while AMPK-dependent signals are suppressed in the liver in lipodystrophy. Central injection of leptin also increased hepatic AMPK activities, but leptin stimulation did not activate AMPK in primary hepatocytes. Furthermore, the hepatic AMPK activation by leptin was completely inhibited by chemical sympathectomy or alpha1 blockers, while neither hepatic vagotomy nor beta blockers affected the activation. Therefore, leptin is suggested to centrally regulate hepatic metabolism using AMPK through alpha-adrenergic effects of sympathetic nervous system. The suppression of hepatic AMPK-dependent signals is supposed to be a cause of metabolic disorders in lipodystrophy, which could be ameliorated by leptin.

3-S-17-2 Orexin system regulating glucose homeodynamics: a potential target for chronotherapy of type 2 diabetes

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Glucose metabolism is dynamically regulated to maintain whole-body glucose homeostasis, according to daily sleep/wake cycle. The hypothalamus is the central region to regulate the glucose homeodynamics, although their mechanisms had long been uncertain. We found that orexin, a hypothalamic neuropeptide, plays central role in this regulation of blood glucose, in addition to the well-established role in stabilization of wakefulness. In this process, orexin bidirectionally regulated hepatic glucose production by changing autonomic nerve balance, thereby promoting generation of daily rhythm in blood glucose in mice. This daily rhythm in blood glucose was beneficial for preventing endoplasmic reticulum stress in the liver, thereby preventing hepatic insulin resistance under pathophysiological conditions, such as aging, obesity, and depression. Moreover, when daily rhythmic action of orexin was amplified by an awake-state administration of nicotine, a stimulator of the orexin system, or a sleeping-state administration of suvorexant, an orexin antagonist, in type 2 diabetic db/db mice, the abnormal glucose tolerance was ameliorated. Therefore, hypothalamic orexin system appears to be a target for chronotherapy against type 2 diabetes.

3-S-17-3 Elucidation of the pathophysiological mechanisms and drug targets for leptin resistance in the CNS

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Obesity is one of the risk factor of diseases such as diabetes, hypertension and hyperlipidemia. Leptin is an anti-obesity hormone, which was discovered in 1994 by Dr. Friedman's group. Leptin exerts its anti-obesity effect through Ob-Rb leptin receptor expressed in hypothalamic neurons. Although leptin has anti-obesity effect, recent evidence suggested that most of the obese patients are in the state of leptin resistance. Therefore, elucidation of the pathophysiological mechanisms of leptin resistance may give us the useful information for the treatment. Previously, we found that ER stress may be involved in the development of leptin resistance and flurubiprofen may be able to ameliorate the resistance (Mol. Pharm., 2008, 74 : 1610-9 ; EMBO Mol. Med., 2014, 6 : 335-46). In the present symposium, we are planning to show other new possible mechanisms of the development of leptin resistance in the CNS. Furthermore, we will show the role of glial cells on the leptin's action in the CNS, which may help to understand the mechanisms of the pathophysiology of leptin resistance.

3-S-17-4 Novel molecular mechanisms of brown rice-derived bioactive substance on anti-obesity and anti-diabetic properties

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Our clinical study has shown that brown rice improves obesity and glucose dysmetabolism in middle-aged men (Br J Nutr 111 : 310, 2014). However, the underlying molecular mechanism remains unclear. We discovered that γ-oryzanol (Orz), a major bioactive component of brown rice, decreases hypothalamic endoplasmic reticulum (ER) stress in high fat diet (HFD)-fed mice, thereby attenuating preference for the dietary fat (Diabetes 61 : 3084, 2012). We recently demonstrated the metabolically-beneficial impact of Orz on pancreatic islet function (Endocrinology 156 : 1242, 2015, Br J Pharmacol 172 : 4519, 2015). In murine isolated islets, Orz enhanced glucose-stimulated insulin secretion (GSIS) via suppression of local dopamine D2 receptor (D2R) signaling. We demonstrated that D2R is confined to β-cells and decreases cAMP levels, thereby decreasing GSIS. In islets from HFD-fed mice, expression levels of D2R signaling molecules were significantly increased, which reciprocally decreased by Orz. HFD-induced ER stress in β-cells aggravates GSIS, leading to apoptosis. We also found that Orz reduced ER stress in islets from HFD-fed mice. Taken together, our data provide rationale of Orz as a potential anti-obesity and anti-diabetic agent.